ADVERSE IMPACTS OF TRANSGENIC CROPS/FOODS-
A Compilation Of Scientific References With Abstracts



Coalition for a GM-Free India
March 2013

Coalition for a GM-Free India is a broad national network of organizations, scientists, farmer unions, consumer groups and individuals committed to keep the food and farms in India free of Genetically Modified Organisms and to protecting India's food security and sovereignty.

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ADVERSE IMPACTS OF TRANSGENIC CROPS/FOODS

A COMPILATION OF SCIENTIFIC REFERENCES WITH ABSTRACTS

For Private Circulation Only

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for Coalition for a GM-Free India

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Printed by Coalition for a GM-Free India
(with support from INSAF)

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Katwaria Sarai, New Delhi : 110 016.

Foreword

This book is a compilation of numerous scientific references of studies, surveys and analyses that point to various adverse impacts of Genetically Modified (GM) crops and foods. Needless to say, the implications of this living, irreversible technology have to be understood on different fronts (as much as possible, because there is also severe dearth of research, that too on long term implications and from independent sources) by policy makers and individual citizens before GMOs (Genetically Modified Organisms) are released into the environment, given that such deployment would take place on a large scale in agriculture.

This book tries to present a picture of the evidence available on specific fronts like molecular level instability and unpredictability induced by the process of GE; health implications flowing out of individual genes used as well as the GMO and also because of the chemicals used along with particular GMOs; environmental implications in terms of impacts on biodiversity, soil, on pests and diseases, impacts on non-target organisms, creation of "super weeds" etc. Some of the studies which have looked at yield myths related to GM crops have also been included. Further, studies that have looked at regulation and suggested improvements in biosafety assessment regimes are part of this compilation. Ethical and Socio-economic implications of GMOs including building up of seed monopolies into the hands of a few multinational corporations are also covered in a few studies presented here. This is by no means an exhaustive compilation, but is only illustrative. In a few cases, we also included some news articles especially when data and information was presented therein.

We hope that this compilation compels sceptics to appreciate the overwhelming evidence that already exists against this technology and also our scientists and regulators to undertake assessments that consist of sound protocols and designs so that early warnings can be captured for appropriate decision-making.

- Coalition for a GM-Free India

ADVERSE IMPACTS OF TRANSGENIC CROPS/FOODS

CONTENTS

IMPRECISION & UNPREDICTABILITY OF SCIENCE & TECHNOLOGY OF GENETIC ENGINEERING

1. **Zhi Liu, Yunhe Li, Jie Zhao, Xiuping Chen, Guiliang Jian, Yufa Peng, and Fangjun Qi (2012): Differentially Expressed Genes Distributed Over Chromosomes and Implicated in Certain Biological Processes for Site Insertion Genetically Modified Rice Kemingdao. Int J Biol Sci. 8(7): 953963.**

Release of genetically modified (GM) plants has sparked off intensive debates worldwide partly because of concerns about potential adverse unintended effects of GM plants to the agro system and the safety of foods. In this study, with the aim of revealing the molecular basis for unintended effects of a single site insertion GM Kemingdao (KMD) rice transformed with a synthetic cry1Ab gene, and bridging unintended effects of KMD rice through clues of differentially expressed genes, comparative transcriptome analyses were performed for GM KMD rice and its parent rice of Xiushui11 (XS11). The results showed that 680 differentially expressed transcripts were identified from 30-day old seedlings of GM KMD rice. The absolute majority of these changed expression transcripts dispersed and located over all rice chromosomes, and existed physical distance on chromosome from the insertion site, while only two transcripts were found to be differentially expressed within the 21 genes located within 100 kb up and down-stream of the insertion site. Pathway and biology function analyses further revealed that differentially expressed transcripts of KMD rice were involved in certain biological processes, and mainly implicated in two types of pathways. One type was pathways implicated in plant stress/defense responses, which were considerably in coordination with the reported unintended effects of KMD rice, which were more susceptible to rice diseases compared to its parent rice XS11; the other type was pathways associated with amino acids metabolism. With this clue, new unintended effects for changes in amino acids synthesis of KMD rice leaves were successfully revealed. Such that an actual case was firstly provided for identification of unintended effects in GM plants by comparative transciptome analysis.

2. Love AJ, C Geri, J Laird, C Carr, BW Yun, GJ Loake et al (2012): Cauliflower Mosaic Virus Protein P6 Inhibits Signaling Responses to Salicylic Acid and Regulates Innate Immunity. PLoS One. 7(10): e47535.

Cauliflower mosaic virus (CaMV) encodes a multifunctional protein P6 that is required for translation of the 35S RNA and also acts as a suppressor of RNA silencing. Here we demonstrate that P6 additionally acts as a pathogenicity effector of an unique and novel type, modifying NPR1 (a key regulator of salicylic acid (SA)- and jasmonic acid (JA)-dependent signaling) and inhibiting SA-dependent defence responses We find that that transgene-mediated expression of P6 in Arabidopsis and transient expression in Nicotiana benthamiana has profound effects on defence signaling, suppressing expression of representative SA-responsive genes and increasing expression of representative JA-responsive genes. Relative to wild-type Arabidopsis P6-expressing transgenics had greatly reduced expression of PR-1 following SA-treatment, infection by CaMV or inoculation with an avirulent bacterial pathogen Pseudomonas syringae pv tomato (Pst). Similarly transient expression in Nicotiana benthamiana of P6 (including a mutant form defective in translational transactivation activity) suppressed PR-1a transcript accumulation in response to Agrobacterium infiltration and following SA-treatment. As well as suppressing the expression of representative SA-regulated genes, P6-transgenic Arabidopsis showed greatly enhanced susceptibility to both virulent and avirulent Pst (titres elevated 10 to 30-fold compared to non-transgenic controls) but reduced susceptibility to the necrotrophic fungus Botrytis cinerea. Necrosis following SA-treatment or inoculation with avirulent Pst was reduced and delayed in P6-transgenics. NPR1 an important regulator of SA/JA crosstalk, was more highly expressed in the presence of P6 and introduction of the P6 transgene into a transgenic line expressing an NPR1:GFP fusion resulted in greatly increased fluorescence in nuclei even in the absence of SA. Thus in the presence of P6 an inactive form of NPR1 is mislocalized in the nucleus even in uninduced plants. These results demonstrate that P6 is a new type of pathogenicity effector protein that enhances susceptibility to biotrophic pathogens by suppressing SA- but enhancing JA-signaling responses.

3. Podevin N and du Jardin P (2012): Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants. GM Crops and Food. 3 (4): 296-300

Multiple variants of the Cauliflower mosaic virus 35S promoter (P35S) are used to drive the expression of transgenes in genetically modified plants, for both research purposes and commercial applications. The genetic organization of the densely packed genome of this virus results in sequence overlap between P35S and viral gene VI, encoding the multifunctional P6 protein. The present paper investigates whether introduction of P35S variants by genetic transformation is likely to result in the expression of functional domains of the P6 protein and in potential impacts in transgenic plants. A bioinformatic analysis was performed to assess the safety for human and animal health of putative translation products of gene VI overlapping P35S. No relevant similarity was identified between the putative peptides and known allergens and toxins, using different databases. From a literature study it became clear that long variants of the P35S do contain an open reading frame, when expressed, might result in unintended phenotypic changes. A flowchart is proposed to evaluate possible unintended effects in plant transformants, based on the DNA sequence actually introduced and on the plant phenotype, taking into account the known effects of ectopically expressed P6 domains in model plants.

4. D. Blaise and K R Kranthi (2011): Cry1Ac expression in transgenic Bt cotton hybrids is influenced by soil moisture and depth. Current Science, Vol 101 (6)

Cry1Ac toxin concentration was assessed in leaves of Bt transgenic cotton hybrid grown on shallow (<60 cm) and deep (>90 cm) black soils of Nagpur, Maharashtra, India. Cry toxin concentration increased up to 80 days after sowing followed by a steep decline. In general, toxin concentration was greater on the deep black soils than the shallow soil. This was because of greater water-holding capacity of the deep soils. Cry toxin concentration was closely related to the soil water content. Beyond (excess moisture) and below (moisture deficit) field capacity, toxin concentration declined. A cubic polynomial best described the relationship between Cry toxin concentration and soil moisture content ($R^2 = 0.95$).

5. Rawat Preeti, Amarjeet Kumar, Krishna Ray, Bhupendra Chaudhary, Sanjeev Kumar, Taru Gautam, Shaveta Kanoria, Gurpreet Kaur, Paritosh Kumar, Deepak Pental & Pradeep Kumar Burma (2011): Detrimental effect of expression of Bt endotoxin Cry1Ac on in vitro regeneration, in vivo growth and development of tobacco and cotton transgenics. Journal of Biosciences 36(2), June 2011, 363376.

High levels of expression of the cry1Ac gene from Bacillus thuringiensis cannot be routinely achieved in transgenic plants despite modifications made in the gene to improve its expression. This has been attributed to the instability of the transcript in a few reports. In the present study, based on the genetic transformation of cotton and tobacco, we show that the expression of the Cry1Ac endotoxin has detrimental effects on both the in vitro and in vivo growth and development of transgenic plants. A number of experiments on developing transgenics in cotton with different versions of cry1Ac gene showed that the majority of the plants did not express any Cry1Ac protein. Based on Southern blot analysis, it was also observed that a substantial number of lines did not contain the cry1Ac gene cassette although they contained the marker gene nptII. More significantly, all the lines that showed appreciable levels of expression were found to be phenotypically abnormal. Experiments on transformation of tobacco with different constructs expressing the cry1Ac gene showed that in vitro regeneration was inhibited by the encoded protein. Further, out of a total of 145 independent events generated with the different cry1Ac gene constructs in tobacco, only 21 showed expression of the Cry1Ac protein, confirming observations made in cotton that regenerants that express high levels of the Cry1Ac protein are selected against during regeneration of transformed events. This problem was circumvented by targeting the Cry1Ac protein to the chloroplast, which also significantly improved the expression of the protein.

6. Jiao, Z., Si, X.X., Li, G.K., Zhang, Z.M., Xu, X.P. (2010): Unintended compositional changes in transgenic rice seeds (Oryza sativa L.) studied by spectral and chromatographic analysis coupled with chemometrics methods. J. Agric. Food Chem. 58, 17461754.

Unintended compositional changes in transgenic rice seeds were studied by near-infrared reflectance, GC-MS, HPLC, and ICP-AES coupled with chemometrics strategies. Three kinds of transgenic rice with resistance to fungal diseases or insect pests were comparatively studied with the nontransgenic counterparts in terms of key nutrients such as protein, amino acids, fatty acids, vitamins, elements, and antinutrient phytic acid recommended by the Organization for Economic Co-operation and Development (OECD). The compositional profiles were discriminated by chemometrics methods, and the discriminatory compounds were protein, three amino acids, two fatty acids, two vitamins, and several elements. Significance of differences for these compounds was proved by analysis of variance, and the variation extent ranged from 20 to 74% for amino acids, from 19 to 38% for fatty acids, from 25 to 57% for vitamins, from 20 to 50% for elements, and 25% for protein, whereas phytic acid content did not change significantly. The unintended compositional alterations as well as unintended change of physical characteristic in transgenic rice compared with nontransgenic rice might be related to the genetic transformation, the effect of which needs to be elucidated by additional studies.

http://www.ncbi.nlm.nih.gov/pubmed/20050687

7. Zeller SL, Kalinina O, Brunner S, Keller B and Schmid B (2010): Transgene X environment interactions in genetically modified wheat. PLoS One. Jul 12; 5(7):e11405

The introduction of transgenes into plants may cause unintended phenotypic effects which could have an impact on the plant itself and the environment. Little is published in the scientific literature about the interrelation of environmental factojrs and possible unintended effects in genetically modified (GM) plants. We studied transgenic bread wheat Triticum aestivum lines expressing the wheat Pm3b gene against the fungus powdery mildew Blumeria graminis f.sp. tritici. Four independent offspring pairs, each consisting of a GM line and its corresponding non-GM control line, were grown under different soil nutrient conditions and with and without fungicide treatment in the glasshouse. Furthermore, we performed a field experiment with a similar design to validate our glasshouse results. The transgene increased the resistance to powdery mildew in all environments. However, GM plants reacted sensitive to fungicide spraying in the glasshouse. Without fungicide treatment, in the glasshouse GM lines had increased vegetative biomass and seed number and a twofold yield compared with control lines. In the field these results were reversed. Fertilization generally increased GM/control differences in the glasshouse but not in the field. Two of four GM lines showed up to 56% yield reduction and a 40-fold increase of infection with ergot disease Claviceps purpurea compared with their control lines in the field experiment; one GM line was very similar to its control. Our results demonstrate that, depending on the insertion event, a particular transgene can have large effects on the entire phenotype of a plant and that these effects can sometimes be reversed when plants are moved from the glasshouse to the field. However, it remains unclear which mechanisms underlie these effects and how they may affect concepts in molecular plant breeding and plant evolutionary ecology.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0011405

8. Latham JR, and AK Wilson (2008): Trans-complementation and Synergism in Plants: Implications for Viral Transgenes?. Molecular Plant Pathology 9: 85-103.

In plants, viral synergisms occur when one virus enhances infection by a distinct or unrelated virus. Such synergisms may be uni-directional or mutualistic but, in either case, synergism implies that protein(s) from one virus can enhance infection by another. A mechanistically related phenomenon is transcomplementation, in which a viral protein, usually expressed from a transgene, enhances or supports the infection of a virus from a distinct species. To gain an insight into the characteristics and limitations of these helper functions of individual viral genes, and to assess their effects on the plantpathogen relationship, reports of successful synergism and transcomplementation were compiled from the peer-reviewed literature and combined with data from successful viral gene exchange experiments. Results from these experiments were tabulated to highlight the phylogenetic relationship between the helper and dependent viruses and, where possible, to identify the protein responsible for the altered infection process. The analysis of more than 150 publications, each containing one or more reports of successful exchanges, trans-complementation or synergism, revealed the following: (i) diverse viral traits can be enhanced by synergism and transcomplementation; these include the expansion of host range, acquisition of mechanical transmission, enhanced specific infectivity, enhanced cell-to-cell and long-distance movement, elevated or novel vector transmission, elevated viral titre and enhanced seed transmission; (ii) transcomplementation and synergism are mediated by many viral proteins, including inhibitors of gene silencing, replicases, coat proteins and movement proteins; (iii) although more frequent between closely related viruses, transcomplementation and synergism can occur between viruses that are phylo-genetically highly divergent. As indicators of the interoperability of viral genes, these results are of general interest, but they can also be applied to the risk assessment of transgenic crops expressing viral proteins. In particular, they can contribute to the identification of potential hazards, and can be used to identify data gaps and limitations in predicting the likelihood of transgene-mediated transcomplementation.

http://www.ncbi.nlm.nih.gov/pubmed/18705887

9. Zolla, L., Rinalducci, S., Antonioli, P., Righetti, P.G. (2008): Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. Journal of Proteome Research 7, 18501861.

To improve the probability of detecting unintended side effects during maize gene manipulations by bombardment, proteomics was used as an analytical tool complementary to the existing safety assessment techniques. Since seed proteome is highly dynamic, depending on the species variability and environmental influence, we analyzed the proteomic profiles of one transgenic maize variety (event MON 810) in two subsequent generations (T05 and T06) with their respective isogenic controls (WT05 and WT06). Thus, by comparing the proteomic profiles of WT05 with WT06 we could determine the environmental effects, while the comparison between WT06 and T06 seeds from plants grown under controlled conditions enabled us to investigate the effects of DNA manipulation. Finally, by comparison of T05 with T06 seed proteomes, it was possible to get some indications about similarities and differences between the adaptations of transgenic and isogenic plants to the same strictly controlled growth environment. Approximately 100 total proteins resulted differentially modulated in the expression level as a consequence of the environmental influence (WT06 vs WT05), whereas 43 proteins resulted up- or down-regulated in transgenic seeds with respect to their controls (T06 vs WT06), which could be specifically related to the insertion of a single gene into a maize genome by particle bombardment. Transgenic seeds responded differentially to the same environment as compared to their respective isogenic controls, as a result of the genome rearrangement derived from gene insertion. To conclude, an exhaustive differential proteomic analysis allows to determine similarities and differences between traditional food and new products (substantial equivalence), and a case-by-case assessment of the new food should be carried out in order to have a wide knowledge of its features.

http://www.ncbi.nlm.nih.gov/pubmed/18393457

10. Rosati A, Bogani P, Santarlasci A, Bulatti M (2008): Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. Plant Mol Biol. 67 (3): 271-81.

The construct inserted in YieldGard MON810 maize, produced by Monsanto, contains the CaMV 35S promoter, the hsp70 intron of maize, the cryI(A)b gene for resistance to lepidopterans and the NOS terminator. In a previous work a truncation event at the 3' end of the cryI(A)b gene leading to the complete loss of the NOS terminator was demonstrated. The 3' maize genome junction region was isolated in the same experiment not showing any homology with known sequences. The aim of the experiments here reported was therefore to isolate and characterize a larger portion of the 3' integration junction from genomic DNA of two commercial MON810 maize lines. Specific primers were designed on the 3' integration junction sequence for the amplification of a 476 bp fragment downstream of the sequence previously detected. In silico analysis identified the whole isolated 3' genomic region as a gene putatively coding for the HECT E3 ubiquitin ligase. RT-PCR performed in this region produced cDNA variants of different length. In silico translation of these transcripts identified 2 and 18 putative additional aminoacids in different variants, all derived from the adjacent host genomic sequences, added to the truncated CRY1A protein. These putative recombinant proteins did not show homology with any known protein domains. Our data gave new insights on the genomic organization of MON810 in the YieldGard maize and confirmed the previous suggestion that the integration in the genome of maize caused a complex recombination event without, apparently, interfering with the activity of the partial CRY1A endotoxin and both the vigor and yield of the YieldGard maize.

http://www.ncbi.nlm.nih.gov/pubmed/18306044

11. Aguilera M, Querci M, Balla B, Prospero A, Ermolli M, Van den Eede G (2008): A Qualitative Approach for the Assessment of the Genetic Stability of the MON 810 Trait in Commercial Seed Maize Varieties. Food Anal. Methods (2008) 1:252258

Maize MON 810 is one of the European Union's (EU) authorized genetically modified organisms (GMO) for placing on the food and feed market. The total number of MON 810 varieties registered in the European Common Catalogue of varieties of agricultural plant species has almost tripled since 2005. One of the requirements described in EU legislation, namely the genetic stability of GM seed varieties, was thus assessed by analyzing the intactness of the entire MON 810 integration and its genotypic stability in commercial varieties available on the market for at least the last 2 years. A combined strategy using qualitative analytical methods made possible to determine the presence/absence of the individual genetic elements and of the whole GM construct. The restriction fragment length polymorphism patterns obtained from amplified whole constructs by long polymerase chain reaction (PCR) were compared side by side. CryIA(b) protein expression levels were determined by enzyme-linked immunosorbent assay. Twenty-four out of the 26 analyzed varieties met the expected stability features. One variety gave negative results in all assays, and one variety contained the necessary genetic elements for expressing CryIA(b) protein although giving negative results for the long PCR product. To our knowledge, this study is the first post-marketing stability analysis performed on GM commercial seed varieties.

http://pubget.com/paper/pgtmp_ef5d2d1aab82f22495e54c4205f6171f/A_Qualitative_Approach_for_the_Assessment_of_the_Genetic_Stability_of_the_MON_810_Trait_in_Commercial_Seed_Maize_Varieties

12. Dong HZ and Li WJ (2007): Variability of endotoxin expression in Bt transgenic cotton. J. Agron. Crop Sci. 193 (1), 2129.

Transgenic cotton expressing Bt (Bacillus thuringiensis) toxins is currently cultivated on a large commercial scale in many countries, but observations have shown that it behaves variably in toxin efficacy against target insects under field conditions. Understanding of the temporal and spatial variation in efficacy and the resulting mechanisms is essential for cotton protection and production. In this review, we summarize current knowledge on variability in Bt cotton efficacy, in particular on the induced variability by environmental stresses. We also discuss the resulting mechanisms and the countermeasures for the inconsistence in efficacy in Bt cotton. It is indicated that insecticidal protein content in Bt cotton is variable with plant age, plant structure or under certain environmental stresses. Variability in Bt cotton efficacy against target insect pests is mainly attributed to the changes in Bt protein content, but physiological changes associated with the production of secondary compounds in plant tissues may also play an important role. Reduction of Bt protein content in late-season cotton could be due to the overexpression of Bt gene at earlier stages, which leads to gene regulation at post-transcription levels and consequently results in gene silencing at a later stage. Methylation of the promotor may be also involved in the declined expression of endotoxin proteins. As a part of total protein, the insecticidal protein in plant tissues changes its level through inhibited synthesis, degradation or translocation to developing plant parts, particularly under environmental stresses, thus being closely correlated to N metabolism. It can be concluded that developing new cotton varieties with more powerful resistance, applying certain plant growth regulators, enhancing intra-plant defensive capability, and maintenance of general health of the transgenic crop are important in realizing the full transgenic potential in Bt cotton.

http://onlinelibrary.wiley.com/doi/10.1111/j.1439037X.2006.00240.x/Abstract;jsessionid=0F0C976C5C24DC2D2C63F3A716ED1061.d01t04?deniedAccessCustomisedMessage=&userIsAuthenticated=false

13. Wilson AK, JR Latham and RA Steinbrecher (2006): Transformation-induced mutations in transgenic plants: Analysis and biosafety implications. Biotechnology and Genetic Engineering Reviews 23: 209-234.

Plant transformation has become an essential tool for plant molecular biologists and, almost simultaneously, transgenic plants have become a major focus of many plant breeding programs. The first transgenic cultivar arrived on the market approximately 15 years ago, and some countries have since commercially approved or deregulated (e.g. the United States) various commodity crops with the result that certain transgenic crop plants, such as herbicide resistant canola and soya and pest resistant maize, are currently grown on millions of acres. Advocates for the use of genetic engineering as a plant breeding tool claim its precision provides a major advantage over other plant breeding techniques. The presumption is that genetic engineering results in (1) only specific and known genotypic changes to the engineered plant (the simple insertion of a defined DNA sequence - the transgene) and (2) only known and specific phenotypic changes [the intended trait(s) encoded by the transgene]. This presumption has strongly influenced biosafety regulation. Regulators typically assume that the plant transformation methods used to introduce a transgene into the plant genome are mostly irrelevant to the risk assessment process and that the major source of risk in transgenic crop plants arises from the transgene itself. The focus of this review is a scientific assessment of the precision of current crop plant transformation techniques.

14. Manetti C, Bianchetti C, Casciani L, Castro C, Di Cocco ME, Miccheli A, Motto M, Conti F (2006): A metabonomic study of transgenic maize (Zea mays) seeds revealed variations in osmolytes and branched amino acids. J Exp Bot 57 (11):2613-2625

The aim of the research was to investigate metabolic variations associated with genetic modifications in the grains of Zea mays using metabonomic techniques. With this in mind, the non-targeted characteristic of the technique is useful to identify metabolites peculiar to the genetic modification and initially undefined. The results obtained showed that the genetic modification, introducing Cry1Ab gene expression, induces metabolic variations involving the primary nitrogen pathway. Concerning the methodological aspects, the experimental protocol used has been applied in this field for the first time. It consists of a combination of partial least square-discriminant analysis and principal component analysis. The most important metabolites for discrimination were selected and the metabolic correlations linking them are identified. Principal component analysis on selected signals confirms metabolic variations, highlighting important details about the changes induced on the metabolic network by the presence of a Bt transgene in the maize genome.

http://www.ncbi.nlm.nih.gov/pubmed/16831843

15. Wilson, A.K., Latham, J.R., Steinbrecher, R.A. (2006): Transformation-induced mutations in transgenic plants: Analysis and biosafety implications. Biotechnology and Genetic Engineering Reviews 23, 209234.

Transgene insertion is infrequently, if ever, a precise event and it therefore causes various alterations to the plant genome. Mutations present at transgene insertion sites include insertion of superfluous DNA and deletion and rearrangement of host chromosomal DNA. These mutations vary in frequency depending in particular on the method of delivery. Transgene insertion sites introduced using Agrobacterium tumefaciens tend to be simpler but can be associated with very large chromosomal rearrangements, while transgenes delivered by particle bombardment appear invariably to be associated with deletion and extensive scrambling of inserted and chromosomal DNA. Nevertheless, the frequency and impact of these mutations are poorly understood, especially those caused by particle bombardment. This is exemplified by the fact that only a single functional transgene insertion site resulting from particle bombardment has been comprehensively analysed for insertion-site mutations. Additionally, most data on insertion-site mutations come from the model organism Arabidopsis thaliana, and it is not yet clear if there are important species-specific differences in patterns of insertion-site mutation. Genetic mutations, linked and unlinked to the transgene insertion-site, also arise from procedures associated with plant transformation, such as tissue culture and infection with A. tumefaciens. These genome-wide mutations can number from hundreds to many thousands per diploid genome, and are likely to be important sources of phenotypic variation. The potential phenotypic consequences of genetic damage from insertion-site mutations and genome-wide mutations are discussed and recommendations for safety assessments are made. A better understanding of the genetic consequences of plant transformation should improve the quality and interpretation of scientific experiments that rely on plant transformation and should advance the debate on the safety of transgenic crops.

http://www.bioscienceresource.org/publications/scientific-publications/

16. Latham, J.R. Wilson, A.K., Steinbrecher, R.A. (2006): The mutational consequences of plant transformation. J. of Biomedicine and Biotechnology 2006, 17.

Plant transformation is a genetic engineering tool for introducing transgenes into plant genomes. It is now being used for the breeding of commercial crops. A central feature of transformation is insertion of the transgene into plant chromosomal DNA. Transgene insertion is infrequently, if ever, a precise event. Mutations found at transgene insertion sites include deletions and rearrangements of host chromosomal DNA and introduction of superfluous DNA. Insertion sites introduced using Agrobacterium tumefaciens tend to have simpler structures but can be associated with extensive chromosomal rearrangements, while those of particle bombardment appear invariably to be associated with deletion and extensive scrambling of inserted and chromosomal DNA. Ancillary procedures associated with plant transformation, including tissue culture and infection with A tumefaciens, can also introduce mutations. These genome-wide mutations can number from hundreds to many thousands per diploid genome. Despite the fact that confidence in the safety and dependability of crop species rests significantly on their genetic integrity, the frequency of transformation-induced mutations and their importance as potential biosafety hazards are poorly understood.

http://www.hindawi.com/journals/bmri/2006/025376/abs/

17. Huixia Wu, Caroline A Sparks and Huw D Jones (2006): Characterisation of T-DNA loci and vector backbone sequences in transgenic wheat produced by Agrobacterium-mediated transformation. Mol Breeding 18: 195-208

Detailed molecular characterisation of transgene loci is a requirement for gaining regulatory approval for environmental release of genetically modified crops. In cereals, it is generally accepted that Agrobacterium-mediated transformation generates cleaner transgene loci with lower copy number and fewer rearrangements than those generated by biolistics. However, in wheat there has been little detailed analysis of T-DNA insertions at genetic and molecular level. Wheat lines transformed using Agrobacterium tumefaciens with bar and gusA (GUS) genes were subjected to genetic and molecular analysis. Unlike previous studies of transgene loci in wheat, we used functional assays for PAT and GUS proteins, combined with PCR and Southern analysis to detect the presence, copy number, linkage and transmission of two transgenes inserted in the same T-DNA. Thirty-four independent transgenic lines were categorised into three types: type I events (38% of total) where the gusA and bar genes displayed complete genetic linkage, segregating together as a single functional locus at the expected ratio of 3:1; type II events (18%), which possessed two or more transgene loci each containing gusA and bar; and type III events (44%), containing an incomplete T-DNA in which either the gusA or bar gene was lost. Most lines in this last category had lost the bar gene situated near the left T-DNA border. Southern analysis indicated that 30% of all lines possessed a single T-DNA copy containing gusA and bar. However, when data on expression and molecular analysis are combined, only 23% of all lines have single copy T-DNAs in which both gene cassettes are functioning. We also report on the presence of plasmid backbone DNA sequence in transgene loci detected using primer pairs outside the left and right T-DNA borders and within the plasmid selectable marker (NptI) gene. Approximately two thirds of the lines contained some vector backbone DNA, more frequently adjacent to the left border. Taken together, these data imply unstable left border function causing premature T-strand termination or read-through into vector backbone. As far as we are aware, this is the first report revealing near border T-DNA truncation and vector backbone integration in wheat transgenic lines produced by Agrobacterium-mediated transformation.

18. Prescott, V.E., Campbell, P.M., Moore, A., Mattes, J., Rothenberg, M.E., Foster, P.S., Higgins, T.J., Hogan, S.P. (2005): Transgenic expression of bean α-amylase inhibitor in peas results in altered structure and immunogenicity. Journal of Agricultural and Food Chemistry 53, 90239030.

The development of modern gene technologies allows for the expression of recombinant proteins in non-native hosts. Diversity in translational and post-translational modification pathways between species could potentially lead to discrete changes in the molecular architecture of the expressed protein and subsequent cellular function and antigenicity. Here, we show that transgenic expression of a plant protein (alpha-amylase inhibitor-1 from the common bean (Phaseolus vulgaris L. cv. Tendergreen)) in a non-native host (transgenic pea (Pisum sativum L.)) led to the synthesis of a structurally modified form of this inhibitor. Employing models of inflammation, we demonstrated in mice that consumption of the modified alphaAI and not the native form predisposed to antigen-specific CD4+ Th2-type inflammation. Furthermore, consumption of the modified alphaAI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these proteins. Thus, transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants possessing altered immunogenicity.

http://www.ncbi.nlm.nih.gov/pubmed/16277398

19. Rang, A., Linke, B., Jansen, B. (2005): Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. Eur Food Res Technol 220, 43843.

The acreage for genetically modified crops (GMOs)particularly soybeanhas steadily increased since 1996, when the first crop of Roundup Ready soybean (intended for food production) was grown. The Roundup Ready soybean varieties derive from a soybean line into which a glyphosate-resistant enolpyruvylshikimate-3-phosphate-synthase (EPSPS) gene was introduced. The inserted and the flanking regions in Roundup Ready soybean have recently been characterized. It was shown that a further 250-bp fragment of the epsps gene is localized downstream of the introduced nos terminator of transcription, derived from the nopaline synthase gene from Agrobacterium tumefaciens. We examined whether this 250-bp fragment could be of functional importance. Our data demonstrate that at least 150bp of this DNA region are transcribed in Roundup Ready soybean. Transcription of the fragment depends on whether read-through events ignore the nos terminator signal located upstream. Our data also indicate that the read-through product is further processed, resulting in four different RNA variants from which the transcribed region of the nos terminator is completely deleted. Deletion results in the generation of open reading frames which might code for (as yet unknown) EPSPS fusion proteins. The nos terminator is used as a regulatory element in several other GMOs used for food production. This implies that read through products and transcription of RNA variants might be a common feature in these GMOs.

http://www.researchgate.net/publication/225493299_Detection_of_RNA_variants_transcribed_from_the_transgene_in_Roundup_Ready_soybean

20. Windels, P., Taverniers, I., Depicker, A., Van Bockstaele, E., De Loose, M. (2001): Characterisation of the Roundup Ready soybean insert. Eur Food Res Technol 213, 107112.

In this article we describe the isolation and characterisation of the junction between insert DNA and plant DNA in the transgenic Roundup Ready soybean line event 40-3-2. Our results establish that during integration of the insert DNA several rearrangements occurred at the 3' NOS junction and that the genomic plant DNA at the pre-integration site may have been rearranged. These findings highlight the utility of characterising junction regions to fulfil the request for information regarding which DNA sequences have been incorporated in commercialised transgenic lines. Furthermore, the characterisation of junction regions is, in our opinion, the method of choice to support method development for detection and identification of plant biotechnology-derived products.

http://cera-gmc.org/docs/articles/09-090-008.pdf

21. Horvath H, Jensen L G, Wong O T, Kohl E, Ullrich S E, Cochran J, Kannangara C G, von Wettstein D (2001): Stability of transgene expression, field performance and recombination breeding of transformed barley lines, Theoretical and Applied Genetics, Vol 102 (1), 1-11.

Stable inheritance of the transgene, consistent expression and competitive agronomic properties of transgenic crops are important parameters for successful use of the latter. These properties have been analyzed with 18 homozygous transgenic barley lines of the cultivar Golden Promise. The lines originated from three independent primary transformants obtained by the biolistic method with three plasmids containing respectively, the bar gene, the uidA gene and the gene for a protein-engineered heat-stable (1,3-1,4)-β-glucanase. Three production levels of recombinant (3-glucanase were identified in homozygous transgenic T3plants, and these remained constant over a 3-year period. In micro-malting experiments, the heat-stable enzyme reached levels of up to 1.4 µg.mg-1 protein and survived kiln drying at levels of 70-100%. In the field trials of 1997 and 1998 the transgenic lines had a reduced 1000-grain weight as well as variable yield depressions compared to the Golden Promise progenitor. In 1999 large-scale propagations of the lines with the highest recombinant enzyme synthesis during germination and of Golden Promise were studied at three different locations. In an irrigated field transgenic lines yielded approximately 6 t.ha-1 and Golden Promise 7.7 t.ha-1. Cross-breeding was carried out to transfer the transgene into a more suitable genetic background. Crosses of the semi-dwarf ari-e mutant Golden Promise gave rise to the four morphological phenotypes nutans, high erect, erect, and ari-e. Two improvements were achieved: (1) F3 lines homozygous for the expression of heat-stable (1,3-1,4)-β-glucanase were found among lines that were homozygous for each of the four morphological phenotypes; (2) improved 1000-grain weights and yields with respect to those of the original transformants were observed in some F4 lines homozygous for the morphological phenotypes and for the transgene. In the case of a homozygous nutans line, the transgenic plants had a higher 1000-grain weight than those lacking the transgene. Like mutants providing useful output traits, transgenic plants will often have to be improved by relocating the gene into more suitable genotypes.

22. Tax FE, Vernon D M (2001): T-DNA-associated duplication/translocations in Arabidopsis. Implications for mutant analysis and functional genomics. Plant Physiol. 126 (4): 1527-38.

T-DNA insertion mutants have become a valuable resource for studies of gene function in Arabidopsis. In the course of both forward and reverse genetic projects, we have identified novel interchromosomal rearrangements in two Arabidopsis T-DNA insertion lines. Both rearrangements were unilateral translocations associated with the left borders of T-DNA inserts that exhibited normal Mendelian segregation. In one study, we characterized the embryo-defective88 mutation. Although emb88 had been mapped to chromosome I, molecular analysis of DNA adjacent to the T-DNA left border revealed sequence from chromosome V. Simple sequence length polymorphism mapping of the T-DNA insertion demonstrated that a >40-kbp region of chromosome V had inserted with the T-DNA into the emb88 locus on chromosome I. A similar scenario was observed with a prospective T-DNA knockout allele of the LIGHT-REGULATED RECEPTOR PROTEIN KINASE (LRRPK) gene. Whereas wild-type LRRPK is on lower chromosome IV, mapping of the T-DNA localized the disrupted LRRPK allele to chromosome V. In both these cases, the sequence of a single T-DNA-flanking region did not provide an accurate picture of DNA disruption because flanking sequences had duplicated and inserted, with the T-DNA, into other chromosomal locations. Our results indicate that T-DNA insertion lines--even those that exhibit straightforward genetic behavior--may contain an unexpectedly high frequency of rearrangements. Such duplication/translocations can interfere with reverse genetic analyses and provide misleading information about the molecular basis of mutant phenotypes. Simple mapping and polymerase chain reaction methods for detecting such rearrangements should be included as a standard step in T-DNA mutant analysis.

23. Carlos E Coviella, David J W Morgan, John T Trumble (2000): Interactions of Elevated CO2 and Nitrogen Fertilization: Effects on Production of Bacillus thuringiensis Toxins in Transgenic Plants. Environ. Entomol. 29(4): 781-787

Elevated atmospheric CO2 concentrations will cause plants to grow faster, lower nitrogen content per unit of plant tissue, and generate higher carbon to nitrogen (C/N) ratios. We hypothesize that production of transgenic proteins will be reduced, thus reducing the efficiency of Bacillus thuringiensis (Bt) transgenes against insect populations. Commercially available transgenic cotton plants expressing the Cry 1Ac gene from Bt were compared with a near isogenic non-Bt cotton line in a split-plot design with two levels of atmospheric CO2 (ambient, 370 ppm and elevated, 900 ppm) incorporating a 2 2 factorial design with two nitrogen (N) fertilization regimes (low, 30 mg N/kg soil/wk and high, 130 mg N/kg soil/wk), and two levels of Bt (presence or absence). Bioassays using Spodoptera exigua (Hubner) and quantitative enzyme-linked immunosorbent assays for toxin content indicated reduced Bt protein production in elevated CO2. The tendency for test insects to consume more foliage from plants with lower N, caused by the elevated CO2, did not compensate for the reduction in toxin production. N fertilization regime interacted with CO2 concentration, showing that plants growing in N limited systems would produce substantially less toxin. The use of transgenic plants is becoming increasingly important and will continue to be so in the next decades. At the same time, atmospheric CO2 increase will affect the effectiveness of this strategy. These observations have implications not only for agricultural use of transgenic plants, but also for the ecological consequences of transfer of Bt toxins to closely related wild plant genotypes.

24. Svitashev S, Ananiev E, Pawlowski W P, Somers D A (2000): Association of transgene integration sites with chromosome rearrangements in hexaploid oat. Theor Appl Genet 100, 872-880.

Transgene loci in 16 transgenic oat (Avena sativa L.) lines produced by microprojectile bombardment were characterized using phenotypic and genotypic segregation, Southern blot analysis, and fluorescence insitu hybridization (FISH). Twenty-five transgene loci were detected; 8 lines exhibited single transgene loci and 8 lines had 2 or 3 loci. Double FISH of the transgene and oat C- and A/D-genome-specific dispersed and clustered repeats showed no preferences in the distribution of transgene loci among the highly heterochromatic C genome and the A/D genomes of hexaploid oat, nor among chromosomes within the genomes. Transgene integration sites were detected at different locations along individual chromosomes, although the majority of transformants had transgenes integrated into subtelomeric and telomeric regions. Transgene integration sites exhibited different levels of structural complexity, ranging from simple integration structures of two apparently contiguous transgene copies to tightly linked clusters of multiple copies of transgenes interspersed with oat DNA. The size of the genomic interspersions observed in these transgene clusters was estimated from FISH results on prometaphase chromosomes to be megabases long, indicating that some transgene loci were significantly larger than previously determined by Southern blot analysis. Overall, 6 of the 25 transgene loci were associated with rearranged chromosomes. These results suggest that particle bombardment- mediated transgene integration may result from and cause chromosomal breakage and rearrangements.

www.pawlowski.cit.cornell.edu/2000.pdf

25. Kumpatla S P, Chandrasekharan MB, Iyer ML, Li G, Hall TC (1998): Genome intruder scanning and modulation systems and transgene silencing. Trends in Plant Science, Vol 3, Issue 3, March 1998. 97-104.

The widespread occurrence of transgene inactivation in plants and classical cases of silencing of duplicated sequences in fungi suggest that all genomes contain defense systems that are capable of monitoring and manipulating intrusive DNA. Such DNA might be recognized by its structure, its sequence composition relative to that of its genomic environment and possibly by its disruption of normal biochemical functions. Although methylation, especially of repeated sequences, is widely associated with gene inactivation, other attributes, including chromatin modification, may be involved. Elimination of inactivated intrusive DNA (presently best documented for filamentous fungi) may also contribute to genomic defense mechanisms in plants. Stable integration and expression of introduced genes are essential for genetically engineered crops, and thus transformation constructs must be designed to avoid host surveillance processes.

http://www.cell.com/trends/plant-science/Abstract/S1360-1385%2897%2901194-1

26. Pawlowski WP, Somers DA (1996): Transgene inheritance in plants genetically engineered by microprojectile bombardment. Mol Biotechnol. 6 (1): 17-30.

Microprojectile bombardment to deliver DNA into plant cells represents a major breakthrough in the development of plant transformation technologies and accordingly has resulted in transformation of numerous species considered recalcitrant to Agrobacterium- or protoplast-mediated transformation methods. This article attempts to review the current understanding of the molecular and genetic behavior of transgenes introduced by microprojectile bombardment. The characteristic features of the transgene integration pattern resulting from DNA delivery via microprojectile bombardment include integration of the full length transgene as well as rearranged copies of the introduced DNA. Copy number of both the transgene and rearranged fragments is often highly variable. Most frequently the multiple transgene copies and rearranged fragments are inherited as a single locus. However, a variable proportion of transgenic events produced by microprojectile bombardment exhibit Mendelian ratios for monogenic and digenic segregation vs events exhibiting segregation distortion. The potential mechanisms underlying these observations are discussed.

http://www.ncbi.nlm.nih.gov/pubmed/8887358

27. Srivastava V, Anderson OD and David W. ow (1996): Single copy transgenic wheat generated through the resolution of complex integration patterns. PNAS, 96: 1117-21

Genetic transformation of plants often results in multiple copies of the introduced DNA at a single locus. To ensure that only a single copy of a foreign gene resides in the plant genome, we used a strategy based on site-specific recombination. The transformation vector consists of a transgene flanked by recombination sites in an inverted orientation. Regardless of the number of copies integrated between the outermost transgenes, recombination between the outermost sites resolves the integrated molecules into a single copy. An example of this strategy has been demonstrated with wheat transformation, where four of four multiple-copy loci were resolved successfully into single-copy transgenes.

http://www.pnas.org/content/96/20/11117.full

28. Inose T and Murata K (1995): Enhanced accumulation of toxic compound in yeast cells having high glycolytic activity a case study on the safety of genetically engineered yeast. Int Journal of Food Sci. and Tech. Vol. 30, 141-146.

The cellular level of methylglyoxal (MG), a highly toxic 2-oxoaldehyde, in Saccharomyces cerevisiae cells transformed with genes for some of the glycolytic enzymes was determined as an index of the safety of genetically engineered yeast and the level was compared with that in non-transformed control cells. the phosphoglucose isomerase (PGI), phosphofructokinase (PFK) and triosephosphate isomerase (TPI) activities significantly increased in the transformants and were approximately five-, three- and sevenfold higher, respectively, than those in the control. When these transformed cells were used for alcohol fermentation from glucose, they accumulated MG in cells at a level sufficient to induce mutagenicity. These results illustrate that careful thought should be given to the potential metabolic products and their safety when a genetically engineered yeast is applied to food-related fermentation processes.

http://onlinelibrary.wiley.com/doi/10.1111/j.13652621.1995.tb01365.x/Abstract

29. Dougherty W G and Parks T D (1995): Transgenes and gene suppression: telling us something new?. Curr Opin Cell Biol. Vol. 7(3): 399-405.

Transgenes provide unique opportunities to assess the relationship between genotype and phenotype in an organism. In most cases, introduction and subsequent expression of a transgene will increase (with a sense RNA) or decrease (with an antisense RNA) the steady-state level of a specific gene product. However, a number of surprising observations have been made in the course of many transgenic studies. We develop a hypothesis that suggests that many examples of endogenous gene suppression by either antisense or sense transcripts are mediated by the same cellular mechanism.

http://119.93.223.179/ScienceDirect/Current%20Opinion%20in%20Cell%20Biology/07-03/sdarticle_014.pdf

HEALTH IMPACTS

BT TOXIN

1. András Székács and Béla Darvas (2012): Chapter 10: Comparative Aspects of Cry Toxin Usage in Insect Control. In "Advanced Technologies for Managing Insect Pests". Eds. Isaac Ishaaya, Subba Reddy Palli and A Rami Horowitz. Springer Science + Business Media. 195-230.

…..It is of outstanding importance to consider the actual form of the Cry endotoxins within the Bt bioinsecticides, such as Dipel. The crystalline structure of these toxins is stabilized by disulfide bonds, and the crystal structure varies across toxin proteins. Due to the numerous disul fi de bonds per protein molecule, the crystals are quite stable to solubilization at neutral pH. As a result, only a small minority of the toxin protein is immediately bioavailable (solubilizable), the vast majority of the crystal mass being only bioaccessible (temporarily non-bioavailable). Thus, just the solubilizable portion of the toxin content is analyzed promptly by ELISA; the bioaccessible part can be detected by ELISA only upon decomposition of the disul fi de bonds stabilizing the crystal structure. Lastly, a minor part of the entire toxin content in the parasporal bodies is non-bioavailable: this amount of endotoxin is decomposed during the process of breaking the crystal structure, and therefore, cannot exert its potential biological activity by direct bioavailability or bioaccessibility…..

2. Vázquez-Padrón R.I., L. Moreno-Fierros, L. Neri-Bazán, A.F. Martínez-Gil, G.A. de-la- Riva and R. López-Revilla (2000): Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Brazilian Journal of Medical and Biological Research, 33, 147-155.

The present paper describes important features of the immune response induced by the Cry1Ac protein from Bacillus thuringiensis in mice. The kinetics of induction of serum and mucosal antibodies showed an immediate production of anti-Cry1Ac IgM and IgG antibodies in serum after the first immunization with the protoxin by either the intraperitoneal or intragastric route. The antibody fraction in serum and intestinal fluids consisted mainly of IgG1. In addition, plasma cells producing anti-Cry1Ac IgG antibodies in Peyer's patches were observed using the solid-phase enzyme-linked immunospot (ELISPOT). Cry1Ac toxin administration induced a strong immune response in serum but in the small intestinal fluids only anti-Cry1Ac IgA antibodies were detected. The data obtained in the present study confirm that the Cry1Ac protoxin is a potent immunogen able to induce a specific immune response in the mucosal tissue, which has not been observed in response to most other proteins.

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-79X2000000200002

3. Vázquez-Padrón, Gonzáles-Cabrera, J., García-Tovar, C., Neri-Bazan, L., Lopéz- Revilla, R., Hernández, M., Moreno-Fierro, L. and de la Riva, G. A. (2000): Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem. Biophys. Res. Comm. 271, 5458.

Bacillus thuringiensis (Bt), considered a safe insecticide, produces insecticidal proteins named Cry during sporulation, which possess exceptional immunological properties. In this work using an immunohistochemical test we demonstrated that Cry1Ac protoxin (pCry1Ac) binds to the mucosal surface of the mouse small intestine. Ligand blot assay allowed us to detect, under denaturing conditions, six pCry1Ac-binding polypeptides present in brush border membrane vesicles isolated from the small intestine. Moreover, this protein induced in situ temporal changes in the electrophysiological properties of the mouse jejunum. The data obtained indicate a possible interaction in vivo of Cry proteins with the animal bowel which could induce changes in the physiological status of the intestine.

http://www.ncbi.nlm.nih.gov/pubmed/10777680

4. Vázquez-Padrón RI, Moreno-Fierros L, Neri-Bazan L, de la Riva GA, Lopez-Revilla R.Vazquez et al (1999): Intragastric and intra-peritoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sciences, 64 (21): 1897-1912.

The spore-forming soil bacterium Bacillus thuringiensis produces parasporal inclusion bodies composed by delta-endotoxins also known as Cry proteins, whose resistance to proteolysis, stability in highly alkaline pH and innocuity to vertebrates make them an interesting candidate to carrier of relevant epitopes in vaccines. The purpose of this study was to determine the mucosal and systemic immunogenicity in mice of Cry1Ac protoxin from B. thuringiensis HD73. Crystalline and soluble forms of the protoxin were administered by intraperitoneal or intragastric route and anti-Cry1Ac antibodies of the major isotypes were determined in serum and intestinal fluids. The two forms of Cry1Ac protoxin administered by intraperitoneal route induced a high systemic antibody response, however, only soluble Cry1Ac induced a mucosal response via intragastric. Serum antibody levels were higher than those induced by cholera toxin. Systemic immune responses were attained with doses of soluble Cry1Ac ranging from 0.1 to 100 microg by both routes, and the maximal effect was obtained with the highest doses. High anti-Cry1Ac IgG antibody levels were detected in the large and small intestine fluids from mice receiving the antigen via i.p. These data indicate that Cry1Ac is a potent systemic and mucosal immunogen.
http://europepmc.org/Abstract/MED/10353588#fragment-Abstract

5. Bernstein IL, Bernstein, J.A. , Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z. Selgrade, M.K., Doerfler, D.L., and Seligy, V.L. (1999): Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environmental Health Perspectives. July 1999; 107(7): 575582.

Although health risks to pesticides containing Bacillus thuringiensis (Bt) have been minimal, the potential allergenicity of these organisms has not been evaluated. Therefore, a health survey was conducted in farm workers before and after exposure to Bt pesticides. Farm workers who picked vegetables that required Bt pesticide spraying were evaluated before the initial spraying operation (n = 48) and 1 and 4 months after (n = 32 and 20, respectively). Two groups of low- (n = 44) and medium- (n = 34) exposure workers not directly exposed to Bt spraying were also assessed. The investigation included questionnaires, nasal/mouth lavages, ventilatory function assessment, and skin tests to indigenous aeroallergens and to a variety of Bt spore and vegetative preparations. To authenticate exposure to the organism present in the commercial preparation, isolates from lavage specimens were tested for Bt genes by DNA-DNA hybridization. Humoral immunoglobulin G (IgG) and immunoglobulin E (IgE) antibody responses to spore and vegetative Bt extracts were assayed. There was no evidence of occupationally related respiratory symptoms. Positive skin-prick tests to several spore extracts were seen chiefly in exposed workers. In particular, there was a significant ($p < 0.05$) increase in the number of positive skin tests to spore extracts 1 and 4 months after exposure to Bt spray. The number of positive skin test responses was also significantly higher in high ($p < 0.05$) than in low- or medium-exposure workers. The majority of nasal lavage cultures from exposed workers was positive for the commercial Bt organism, as demonstrated by specific molecular genetic probes. Specific IgE antibodies were present in more high-exposure workers ($p < 0.05$) than in the low and medium groups. Specific IgG antibodies occurred more in the high ($p < 0.05$) than in the low-exposure group. Specific IgG and IgE antibodies to vegetative organisms were present in all groups of workers. Exposure to Bt sprays may lead to allergic skin sensitization and induction of IgE and IgG antibodies, or both.

6. Vázquez RI, Moreno-Fierros L, Neri-Bazan L, De La Riva GA, Lopez-Revilla R. (1999): Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scandinavian Journal of Immunology. 49(6): 578-84.

Recently we demonstrated that recombinant Cry1Ac protoxin from Bacillus thuringiensis is a potent systemic and mucosal immunogen. In this study we compared the adjuvant effects of Cry1Ac and cholera toxin (CT) for the hepatitis B surface antigen (HBsAg) and bovine serum albumin (BSA). The antibody responses of intestinal secretions and serum were determined by ELISA in Balb/c mice immunized through the intragastric (IG) or intraperitoneal (IP) routes. When HBsAg was administered via IG, the anti-HBsAg intestinal response was not enhanced by either Cry1Ac or CT, whereas via IP Cry1Ac increased the anti-HBsAg intestinal immunoglobulin (Ig)G response and CT increased the intestinal IgA and IgM responses. Serum anti-BSA antibodies increased when BSA was co-administered with CT or Cry1Ac by both routes. Cholera toxin and Cry1Ac co-administered via IP increased the IgG anti-BSA response in fluid of the large intestine and CT also increased the IgA and IgM responses slightly. When co-administered via IP, CT and Cry1Ac did not affect the IgG anti-BSA response of the small intestine significantly. We conclude that Cry1Ac is a mucosal and systemic adjuvant as potent as CT which enhances mostly serum and intestinal IgG antibody responses, especially at the large intestine, and its effects depend on the route and antigen used. These features make Cry1Ac of potential use as carrier and/or adjuvant in mucosal and parenteral vaccines.

http://www.researchgate.net/publication/12945282_Bacillus_thuringiensis_Cry1Ac_protoxin_is_a_potent_systemic_and_mucosal_adjuvant

7. Swadener C. (1994): Bacillus thuringiensis, Insecticide Fact Sheet, Journal of Pesticide reform, Fall 1994, Vol 14, No 3.

As hazards of conventional, broad acting pesticides are documented, researchers look for pesticides that are toxic only to the target pest, have less impact on other species, and have fewer environmental hazards. Bacillus thuringiensis (B.t.) insecticides result from this research. However, there is evidence suggesting that B.t. is not as benign as the manufacturers would like us to believe, and that care is warranted in its use. After the insect ingests B.t., the crystal is dissolved in the insect's alkaline gut. Then the insect's digestive enzymes break clown the crystal structure and activate B.t.'s insecticidal component, called the delta-endotoxin. The delta-endotoxin binds to the cells lining the midgut membrane and creates pores in the membrane, upsetting the gut's ion balance. The insect soon stops feeding and starves to death. If the insect is not susceptible to the direct action of the delta-endotoxin, death occurs after B.t. starts vegetative growth inside the insect's gut. The spore germinates after the gut membrane is broken; it then reproduces and makes more spores. This body-wide infection eventually kills the insect. Since B.t. is a live microbial organism, testing for the possible hazards of B.t. is conducted differently chat for conventional pesticides. Microbial toxicity is described using pathogenicity (the ability of the microbe to cause disease) and infectivity (the ability of the organism to reproduce within the body,) The United States Environmental Protection Agency (EPA) requires no testing of B.t. for carcinogenicity, mutagenicity, or chronic toxicity.

Bacillus thuringiensis var. kurstaki: There have been few experimental studies assessing the toxiciy of B.t.k. to humans. Most information comes from occupational exposures, or from exposures occurring during large-scale B.t.k. programs. 28 Human volunteers suffered from nausea, vomiting, diarrhea, colic-like pains, and fever after eating food contaminated with one B.t. strain, B.t. var. galleriae. These examples indicate the close relationship between B.t. and disease-causing pathogens.

Increased Susceptibility People with compromised immune systems or preexisting allergies may be particularly susceptible to the effects of B.t. Development of resistance occurs faster when larger amounts of a pesticide are used, so that use of crop plants genetically-engineered to produce the B.t. toxin could dramatically increase the number of B.t.-resistant insects.

http://eap.mcgill.ca/MagRack/JPR/JPR_22.htm

GLYPHOSATE & OTHER HERBICIDES

1. Mesnage R., Bernay B., Séralini G-E. (2013, in press). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology.

Pesticides are always used in formulations as mixtures of an active principle with adjuvants. Glyphosate, the active ingredient of the major pesticide in the world, is an herbicide supposed to be specific on plant metabolism. Its adjuvants are generally considered as inert diluents. Since side effects for all these compounds have been claimed, we studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. For this we detailed their compositions and toxicities, and as controls we used a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate. This was performed after 24 h exposures on hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines. We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. The compositions in adjuvants were analyzed by mass spectrometry. Here we demonstrate that all formulations are more toxic than glyphosate, and we separated experimentally three groups of formulations differentially toxic according to their concentrations in ethoxylated adjuvants. Among them, POE-15 clearly appears to be the most toxic principle against human cells, even if others are not excluded. It begins to be active with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3 ppm, at environmental/occupational doses. We demonstrate in addition that POE-15 induces necrosis when its first micellization process occurs, by contrast to glyphosate which is known to promote endocrine disrupting effects after entering cells. Altogether, these results challenge the establishment of guidance values such as the acceptable daily intake of glyphosate, when these are mostly based on a long term in vivo test of glyphosate alone. Since pesticides are always used with adjuvants that could change their toxicity, the necessity to assess their whole formulations as mixtures becomes obvious. This challenges the concept of active principle of pesticides for non-target species.

http://dx.doi.org/10.1016/j.tox.2012.09.006

2. Silvia L. Lopez, Delia Aiassa, Stella Benitez-Leite, Rafael Lajmanovich, Fernando Manas, Gisela Poletta, Norma Sanchez, Maria Fernanda Simoniello, and Andres E. Carrasco (2012): Pesticides Used in South American GMO-Based Agriculture: A Review of Their Effects on Humans and Animal Models. Advances in Molecular Toxicology. The chapter forms part of a new book, Advances in Molecular Toxicology, Vol. 6, published by Elsevier:

In South America, the incorporation of genetically modified organisms (GMO) engineered to be resistant to pesticides changed the agricultural model into one dependent on the massive use of agrochemicals. Different pesticides are used in response to the demands of the global consuming market to control weeds, herbivorous arthropods, and crop diseases. Here, we review their effects on humans and animal models, in terms of genotoxicity, teratogenicity, and cell damage. We also stress the importance of biomarkers for medical surveillance of populations at risk and propose the use of biosensors as sensitive resources to detect undesirable effects of new molecules and environmental pollutants. The compatibility of glyphosate, the most intensively used herbicide associated to GMO crops, with an integrated pest management for soybean crops, is also discussed.

http://www.amazon.com/Advances-Molecular-Toxicology-Volume-6/dp/0444593896

3. Koller VJ, Furhacker M, Nersesyan A, Misik M, Eisenbauer M, Knasmueller S. (2012): Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. Arch Toxicol. 86 (5): 805813.

Glyphosate (G) is the largest selling herbicide worldwide; the most common formulations (Roundup, R) contain polyoxyethyleneamine as main surfactant. Recent findings indicate that G exposure may cause DNA damage and cancer in humans. Aim of this investigation was to study the cytotoxic and genotoxic properties of G and R (UltraMax) in a buccal epithelial cell line (TR146), as workers are exposed via inhalation to the herbicide. R induced acute cytotoxic effects at concentrations > 40 mg/l after 20 min, which were due to membrane damage and impairment of mitochondrial functions. With G, increased release of extracellular lactate dehydrogenase indicative for membrane damage was observed at doses > 80 mg/l. Both G and R induced DNA migration in single-cell gel electrophoresis assays at doses > 20 mg/l. Furthermore, an increase of nuclear aberrations that reflect DNA damage was observed. The frequencies of micronuclei and nuclear buds were elevated after 20-min exposure to 10-20 mg/l, while nucleoplasmatic bridges were only enhanced by R at the highest dose (20 mg/l). R was under all conditions more active than its active principle (G). Comparisons with results of earlier studies with lymphocytes and cells from internal organs indicate that epithelial cells are more susceptible to the cytotoxic and DNA-damaging properties of the herbicide and its formulation. Since we found genotoxic effects after short exposure to concentrations that correspond to a 450-fold dilution of spraying used in agriculture, our findings indicate that inhalation may cause DNA damage in exposed individuals.

4. Antoniou M, Habib MEM, Howard CV, Jennings RC, Leifert C, Nodari RO, Robinson CJ, Fagan J (2012): Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence. J Environ Anal Toxicol S4:006.

The publication of a study in 2010, showing that a glyphosate herbicide formulation and glyphosate alone caused malformations in the embryos of Xenopus laevis and chickens through disruption of the retinoic acid signalling pathway, caused scientific and regulatory controversy. Debate centred on the effects of the production and consumption of genetically modified Roundup Ready® soy, which is engineered to tolerate applications of glyphosate herbicide. The study, along with others indicating teratogenic and reproductive effects from glyphosate herbicide exposure, was rebutted by the German Federal Office for Consumer Protection and Food Safety, BVL, as well as in industry-sponsored papers. These rebuttals relied partly on unpublished industry-sponsored studies commissioned for regulatory purposes, which, it was claimed, showed that glyphosate is not a teratogen or reproductive toxin. However, examination of the German authorities' draft assessment report on the industry studies, which underlies glyphosate's EU authorisation, revealed further evidence of glyphosate's teratogenicity. Many of the malformations found were of the type defined in the scientific literature as associated with retinoic acid teratogenesis. Nevertheless, the German and EU authorities minimized these findings in their assessment and set a potentially unsafe acceptable daily intake (ADI) level for glyphosate. This paper reviews the evidence on the teratogenicity and reproductive toxicity of glyphosate herbicides and concludes that a new and transparent risk assessment needs to be conducted. The new risk assessment must take into account all the data on the toxicity of glyphosate and its commercial formulations, including data generated by independent scientists and published in the peer-reviewed scientific literature, as well as the industry-sponsored studies.

http://www.omicsonline.org/2161-0525/2161-0525-S4-006.php?aid=7453

5. Paganelli, A., Gnazzo, V., Acosta, H., López, S.L., Carrasco, A.E. (2010): Glyphosatebased herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. Chem. Res. Toxicol., August 9.

The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosate-based herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. Xenopus laevis embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in Xenopus embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of otx2 expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.

http://www.ncbi.nlm.nih.gov/pubmed/20695457

6. George, J., Prasad, S., Mahmood, Z., Shukla, Y. (2010): Studies on glyphosate induced carcinogenicity in mouse skin. A proteomic approach. J. of Proteomics 73, 951964.

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu-Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu-Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

http://www.ncbi.nlm.nih.gov/pubmed/20045496

7. Benachour, N., Séralini, G-E. (2009): Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chem. Res. Toxicol. 22(1): 97105.

We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from R formulation-treated crops.

http://www.ncbi.nlm.nih.gov/pubmed/19105591

8. Gasnier C., Dumont C., Benachour N., Clair E., Chagnon M.C., Séralini G-E. (2009): Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262, 184191.

Glyphosate-based herbicides are the most widely used across the world; they are commercialized in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERalpha, ERbeta) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/ mutagens/ reprotoxics is discussed. Web link:

http://www.ncbi.nlm.nih.gov/pubmed/19539684

9. Benitez-Leite, S., Macchi, M.A., Acosta, M. (2009): Malformaciones congénitas asociadas a agrotóxicos. Arch. Pediatr. Drug 80, 237247.

Introduction: exposure to pesticides is a known risk for human health. This paper describes the relationship between parental exposure and congenital malformations in the newborn.

Objective: to study the association between exposure to pesticides and congenital malformations in neonates born in the Regional Hospital of Encarnacion, in the Department of Itapua, Paraguay.

Materials and methods: a prospective case-controlled study carried out from March 2006 to February 2007. Cases included all newborns with congenital malformations, and controls were all healthy children of the same sex born immediately thereafter. Births outside the hospital were not counted. Exposure was considered to be any contact with agricultural chemicals, in addition to other known risk factors for congenital defects.

Results: a total of 52 cases and 87 controls were analyzed. The average number of births each month was 216. The significantly associated risk factors were: living near treated fields (OR 2,46, CI95% 1,09-5,57, p<0,02), dwelling located less than 1 km (OR 2,66, CI95% 1,19-5,97, p<0,008), storage of pesticides in the home (OR 15,35, CI95% 1,96-701,63), p<0,003), direct or accidental contact with pesticides (OR 3,19, CI95% 0,97-11,4, p<0,04), and family history of malformation (OR 6,81, CI95% 1,94-30,56, p<0,001). Other known risk factors for malformations did not show statistical significance.

Conclusion: the results show an association between exposure to pesticides and congenital malformations. Further studies are required to confirm these findings.

10. Mañas, F., Peralta, L., Raviolo, J., Garci, O.H., Weyers, A., Ugnia, L., Gonzalez, C.M., Larripa, I., Gorla, N. (2009): Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety 72, 834837.

Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4 h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48 h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.57.5 mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8 mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200400 mg/kg. AMPA was genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity.

http://www.sciencedirect.com/science/article/pii/S0147651308002662

11. Malatesta, M., Perdoni, F., Santin, G., Battistelli, S., Muller, S., Biggiogerra, M. (2008): Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. Toxicol. in Vitro 22, 18531860.

Previous studies on mice fed genetically modified (GM) soybean demonstrated modifications of the mitochondrial functions and of the transcription/splicing pathways in hepatocytes. The cause(s) of these alterations could not be conclusively established but, since the GM soybean used is tolerant to glyphosate and was treated with the glyphosate-containing herbicide RoundupTM, the possibility exists that the effects observed may be due to herbicide residues. In order to verify this hypothesis, we treated HTC cells with 110 mM Roundup and analysed cellular features by flow cytometry, fluorescence and electron microscopy. Under these experimental conditions, the death rate and the general morphology of HTC cells were not affected, as well as most of the cytoplasmic organelles. However, in HTC-treated cells, lysosome density increased and mitochondrial membranes modified indicating a decline in the respiratory activity. Moreover, nuclei underwent morpho-functional modifications suggestive of a decreased transcriptional/splicing activity. Although we cannot exclude that other factors than the presence of the herbicide residues could be responsible for the cellular modifications described in GM-fed mice, the concordance of the effects induced by low concentrations of Roundup on HTC cells suggests that the presence of Roundup residues could be one of the factors interfering with multiple metabolic pathways.

http://forumrolnictwaekologicznego.pl/cms/images/Ciekawe%20artyku%C5%82y/Malatesta%20Hepatoma%20tissue%202008.pdf

12. Eriksson, M., Hardell, L., Carlberg, M., Akerman, M. (2008): Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. International Journal of Cancer 123,16571663.

We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91 %) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with >10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.

http://www.ncbi.nlm.nih.gov/pubmed/18623080

13. B Cisterna, F Flach, L Vecchio, SML Barabino, S Battistelli, TE Martin, M Malatesta, M Biggiogera (2008): Can a genetically modified organism-containing diet influence embryonic development? A preliminary study on pre-implantation mouse embryos. Cisterna. Vol. 52(4)

In eukaryotic cells, pre-mRNAs undergo several transformation steps to generate mature mRNAs. Recent studies have demonstrated that a diet containing a genetically modified (GM) soybean can induce modifications of nuclear constituents involved in RNA processing in some tissues of young, adult and old mice. On this basis, we have investigated the ultrastructural and immunocytochemical features of pre-implantation embryos from mice fed either GM or non- GM soybean in order to verify whether the parental diet can affect the morpho-functional development of the embryonic ribonucleoprotein structural constituents involved in premRNA pathways. Morphological observations revealed that the general aspect of embryo nuclear components is similar in the two experimental groups. However, immunocytochemical and in situ hybridization results suggest a temporary decrease of pre-mRNA transcription and splicing in 2-cell embryos and a resumption in 4-8-cell embryos from mice fed GM soybean; moreover, pre-mRNA maturation seems to be less efficient in both 2-cell and 4-8-cell embryos from GM-fed mice than in controls. Although our results are still preliminary and limited to the pre-implantation phases, the results of this study encourage deepening on the effects of food components and/or contaminants on embryo development.

14. Paz-y-Miño, C., Sánchez, M.E., Arévalo, M., Muñoz, M.J., Witte, T., De-la- Carrera, G.O., Leone, P. E. (2007): Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. Genetics and Molecular Biology 30, 456-460.

We analyzed the consequences of aerial spraying with glyphosate added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the comet assay. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 ìm) compared to the control group (comet length = 25.94 ìm). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.

15. Martinez A, Reyes I, Reyes N (2007): Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells. Biomedica. 2007 Dec;27(4):594-604.

Glyphosate is a broad-spectrum, non-selective herbicide and commonly used to eliminate weeds in agricultural and forest settings. Studies evaluating glyphosate toxicity in animals and environment show that commercial formulations of glyphosate are more toxic than the active component itself. Technical grade glyphosate was compared with the commercial formulation Roundup in their respective toxicities on human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were exposed to different concentrations of glyphosate, either technical grade or in the form of Roundup for 24 h, 48 h, 72 h, and 96 h. Cytotoxicity was assayed by trypan blue dye exclusion method and reduction of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2Htetrazolium-5-carboxyanilide inner salt)XTT reagent. Both technical grade glyphosate and Roundup formulation were toxic to human peripheral blood mononuclear cells. Cytotoxicity of Roundup was higher than cytotoxicity of glyphosate, since the LC50 (50% lethal concentration) determined by the trypan blue exclusion method at 24 h was the equivalent of 56.4 microg/ml of glyphosate in the form of Roundup and 1,640 microg/ml (1.64 mg/ml) for technical grade glyphosate.This in vitro study confirmed the toxic effects on human cells by glyphosate and its commercial preparations. Commercial formulations were more cytotoxic than the active component alone, supporting the concept that additives in commercial formulations play a role in the toxicity attributed to glyphosate-based herbicides.

16. Dallegrave E, Mantese FD, Oliveira RT, Andrade AJ, Dalsenter PR, Langeloh A (2007). Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch Toxicol. 81(9):665-73.

Glyphosate is the active ingredient and polyoxyethyleneamine is the surfactant present in the herbicide Roundup formulation commercialized in Brazil. The aim of this study was to assess the reproductive effects of glyphosate-Roundup on male and female offspring of Wistar rats exposed during pregnancy and lactation. Dams were treated orally with water or 50, 150 or 450 mg/kg glyphosate during pregnancy (21-23 days) and lactation (21 days). These doses do not correspond to human exposure levels. The results showed that glyphosate-Roundup did not induce maternal toxicity but induced adverse reproductive effects on male offspring rats: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in the serum testosterone level at puberty, and signs of individual spermatid degeneration during both periods. There was only a vaginal canal-opening delay in the exposed female offspring. These findings suggest that in utero and lactational exposure to glyphosate-Roundup may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood.

17. Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., Séralini, G-E. (2007): Time- and dose-dependent effects of Roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology 53, 12633.

Roundup is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD(50)) of Roundup with embryonic cells is 0.3% within 1 h in serum-free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1-2%, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01% (with 210 microM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact

http://www.ncbi.nlm.nih.gov/pubmed/17486286?dopt=Abstract&holding=f1000,f1000m

18. Bellé, R., Le Bouffant, R., Morales, J., Cosson, B., Cormier, P., Mulner-Lorillon, O. (2007): Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development. J. Soc. Biol. 201, 317327.

Cell division is an essential process for heredity, maintenance and evolution of the whole living kingdom. Sea urchin early development represents an excellent experimental model for the analysis of cell cycle checkpoint mechanisms since embryonic cells contain a functional DNA-damage checkpoint and since the whole sea urchin genome is sequenced. The DNA-damaged checkpoint is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism, and for commitment to cell death by apoptosis in the case of failure to repair. New insights in cancer biology lead to two fundamental concepts about the very first origin of cancerogenesis. Cancers result from dysfunction of DNA-damaged checkpoints and cancers appear as a result of normal stem cell (NCS) transformation into a cancer stem cell (CSC). The second aspect suggests a new definition of "cancer", since CSC can be detected well before any clinical evidence. Since early development starts from the zygote, which is a primary stem cell, sea urchin early development allows analysis of the early steps of the cancerization process. Although sea urchins do not develop cancers, the model is alternative and complementary to stem cells which are not easy to isolate, do not divide in a short time and do not divide synchronously. In the field of toxicology and incidence on human health, the sea urchin experimental model allows assessment of cancer risk from single or combined molecules long before any epidemiologic evidence is available. Sea urchin embryos were used to test the worldwide used pesticide Roundup that contains glyphosate as the active herbicide agent; it was shown to activate the DNA-damage checkpoint of the first cell cycle of development. The model therefore allows considerable increase in risk evaluation of new products in the field of cancer and offers a tool for the discovery of molecular markers for early diagnostic in cancer biology. Prevention and early diagnosis are two decisive elements of human cancer therapy.

http://www.ncbi.nlm.nih.gov/pubmed/18157084

19. Soso, A.B., Barcellos, L.J.G., Ranzani-Paiva, M.J., Kreutz, L.K., Quevedo, R.M., Anziliero, D. Lima, M., Silva, L.B.,Ritter, F., Bedin, A.C., Finco, J.A. (2007): Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (Rhamdia quelen). Environmental Toxicology and Pharmacology 23, 308313.

This work was carried out to verify the effect of a glyphosate-based herbicide on Jundiá hormones (cortisol, 17β-estradiol and testosterone), oocyte and swim-up fry production. Earthen ponds containing Jundiá females were contaminated with glyphosate (3.6mg/L); blood samples were collected from eight females from each treatment immediately before, or at 1, 10, 20, 30 and 40 days following contamination. A typical post-stress rise in cortisol levels was observed at the 20th and 40th days following exposure to glyphosate. At the 40th day, 17β-estradiol was decreased in the exposed females. A similar number of oocytes were stripped out from females from both groups; however, a lower number of viable swim-up fry were obtained from the herbicide exposed females, which also had a higher liver-somatic index (LSI). The results indicate that the presence of glyphosate in water was deleterious to Rhamdia quelen reproduction, altering steroid profiles and egg viability.

http://www.ncbi.nlm.nih.gov/pubmed/21783773

20. Hokanson R, Fudge R, Chowdhary R, Busbee D. (2007): Alteration of estrogen regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Hum Exp Toxicol. 2007 Sep; 26 (9): 747-52.

Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized in vitro DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed in utero.

http://www.ncbi.nlm.nih.gov/pubmed/17984146

21. Richard S., Moslemi S., Siphautar H., Benachour N., Séralini G-E. (2005): Differential effects of glyphosate and Roundup on human placental cells and aromatase. Environ Health Perspect 113 (6), 71620.

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

http://www.ncbi.nlm.nih.gov/pubmed/15929894

22. Marc, J., Bellé, R., Morales, J., Cormier, P., Mulner-Lorillon, O. (2004): Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. Toxicological Sciences 82, 436442.

A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B in vivo, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1/cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression.

http://toxsci.oxfordjournals.org/content/82/2/436.full

23. Marc, J., Mulner-Lorillon, O., Bellé, R. (2004): Glyphosate-based pesticides affect cell cycle regulation. Biology of the Cell, 96: 245249.

Cell-cycle dysregulation is a hallmark of tumor cells and human cancers. Failure in the cell-cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide, was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell-cycle adverse concentration.

http://www.ncbi.nlm.nih.gov/pubmed/15182708

24. Haefs, R., Schmitz-Eiberger, M., Mainx, H.G., Mittelstaedt, W., Noga, G. (2002): Studies on a new group of biodegradable surfactants for glyphosate. Pest Manag. Sci. 58, 825833.

The effectiveness of a homologous series of biodegradable rapeseed oil derivatives (triglyceride ethoxylates; Agnique RSO series containing an average of 5, 10, 30 and 60 units of ethylene oxide (EO) as adjuvants for foliage-applied, water-soluble, systemic active ingredients was evaluated employing glyphosate as an example. Previous experiments had revealed that the surfactants used are not phytotoxic at concentrations ranging from 1 to 10 g litre-1. The experiments were performed using Phaseolus vulgaris L and nine selected weed species, grown in a growth chamber at 25/20 (+/- 2) degrees C day/night temperature and 40/70 (+/- 10)% relative humidity. The surfactants were evaluated for enhancement of spray retention, and foliar penetration biological efficacy of glyphosate. Glyphosate was applied at a concentration of 43 mM. The surfactants were added at concentrations of 1 g litre-1. The commercial glyphosate 360 g AE litre-1 SL Roundup Ultra and unformulated glyphosate served as references. The surfactants used improved spray retention, foliar penetration and biological efficacy. Some of the formulations were comparable to the performance of Roundup Ultra in the aspects evaluated; some were even more effective in enhancing spray liquid retention and promoting glyphosate phytotoxicity in several plant species. In these studies Agnique RSO 60 generally was most effective.

http://www.ncbi.nlm.nih.gov/pubmed/12192908

25. Marc, J., Mulner-Lorillon, O., Boulben, S., Hureau, D., Durand, G., Bellé, R. (2002): Pesticide Roundup provokes cell division dysfunction at the level of CDK1/ cyclin B activation. Chem Res Toxicol. 15, 32631.

To assess human health risk from environmental chemicals, we have studied the effect on cell cycle regulation of the widely used glyphosate-containing pesticide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization, which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8% Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2%, while glyphosate alone was ineffective, thus indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, as judged cytologically. Since CDK1/cyclin B regulates universally the M-phase of the cell cycle, we analyzed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B in vivo. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B regulator, our results question the safety of glyphosate and Roundup on human health.

26. Poulsen, M.S., Rytting, E., Mose,T., Knudsen, L.E. (2000): Modeling placental transport: correlation of in vitro BeWo cell permeability and ex vivo human placental perfusion. Toxicol. in Vitro 23, 13801386.

The placental passage of three compounds with different physicochemical properties was recently investigated in ex vivo human placental perfusion experiments (caffeine, benzoic acid, and glyphosate) [Mose, T., Kjaerstad, M.B., Mathiesen, L., Nielsen, J.B., Edelfors, S., Knudsen, L.E., 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. J. Toxicol. Environ. Health, Part A 71, 984-991]. In this work, the transport of these same three compounds, plus the reference compound antipyrine, was investigated using BeWo (b30) cell monolayers. Transport across the BeWo cells was observed in the rank order of caffeine>antipyrine>benzoic acid>glyphosate in terms of both the apparent permeability coefficient and the initial slope, defined as the linear rate of substance transferred to the fetal compartment as percent per time, a parameter used to compare the two experimental models. The results from the in vitro studies were in excellent agreement with the ex vivo results (caffeine approximately antipyrine>benzoic acid>glyphosate). However the transfer rate was much slower in the BeWo cells compared to the perfusion system. The advantages and limitations of each model are discussed in order to assist in the preparation, prediction, and performance of future studies of maternal-fetal transfer.

http://www.ncbi.nlm.nih.gov/pubmed/19647068

27. Hardell, L., Eriksson, M. A. (1999): Case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer 85, 135360.

BACKGROUND: The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.
METHODS: A population-based case-control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Univariate and multivariate analyses were performed with the SAS statistical data program.
RESULTS: Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0-2.5) and fungicides (OR, 3.7; 95% CI, 1.1-13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9-2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0-6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.
CONCLUSIONS: Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.

http://www.ncbi.nlm.nih.gov/pubmed/10189142

28. Andre Leu, 'Glyphosate: A review of its health and environmental effects', in turn based on Caroline Cox, "Glyphosate Fact Sheet", Journal of Pesticide Reform, Vol.18, No. 3, Fall 1998

Glyphosate is widely used in the mistaken belief that it is harmless, safe and readily breaks down leaving no residues. Consequently, it is sprayed in public areas while people are present and by operators without protective clothing. These people are exposed to the drift of this herbicide. The facts show that Glyphosate causes a range of health problems to humans, plants and animals, it causes environmental problems and that it is highly persistent. It is time that the widespread use of this toxic chemical on roadsides, footpaths, parks, gardens, schools, farms, forestry, national parks etc was stopped or highly restricted.

Conclusion extracted from:

http://www.ofa.org.au/papers//glyphosatereview.htm

29. Glyphosate Fact Sheet (1996), Pesticides News No.33, September 1996, p28-29

Acute toxicity: In the UK, glyphosate is the most frequent cause of complaints and poisoning incidents recorded by the Health and Safety Executive's Pesticides Incidents Appraisal Panel (PIAP). Between 1990 and 1995, 33 complaints were received and 34 poisonings recorded including a single death by suicide in 1990(12,13). In California, glyphosate is one of the most commonly reported causes of illness or injury to workers from pesticides. The most common complaints are eye and skin irritation(14). The US authorities have recommended a no re-entry period of 12 hours where glyphosate is used in agricultural or industrial situations. No such recommendation exists in the UK.

Chronic toxicity: Some literature suggests that glyphosate can cause some chronic health effects and birth defects in certain test animals when administered at high doses over prolonged periods(15). Chronic feeding studies have shown reduced weight gain, blood and pancreatic effects, but no evidence of carcinogenicity to humans. A US EPA report says: "Effects on pregnant mothers and foetuses included diarrhoea, decreased weight gain, nasal discharge and death of mothers and kidney and digestive disorders in rat pups"

Conclusion:Glyphosate can be an effective tool in weed control programmes and is relatively less harmful than many of the products which compete with it in the market place. There is nevertheless evidence of toxic effects on humans as well as environmental toxicity, indirect environmental damage and resistance in some target weed species. Since glyphosate is being marketed as a safe and environmentally friendly product and its use is so extensive, there is a danger that damage to non-target plants including endangered species will increase. Habitat damage and destruction will occur more frequently and more instances of weed resistance will appear. Cultivation of glyphosate resistant crops will potentially exacerbate these problems.

So while glyphosate provides a welcome move away from chemicals which are highly toxic to humans and other non target organisms, and from chemicals which cause direct and lasting damage to the environment, it may be introducing more subtle indirect forms of damage of which users need to be aware.

http://www.pan-uk.org/pestnews/Actives/glyphosa.htm

30. Watanabe, T., Iwase, T. (1996): Development and dymorphogenic effects of glufosinate ammonium on mouse embryos in culture. Teratogenesis, carcinogenesis and mutagenesis 16, 287-299.

The effects of glufosinate ammonium on embryonic development in mice were examined using whole embryo and micromass cultures of midbrain and limb bud cells. In day 8 embryos cultured for 48 hr, glufosinate caused significant overall embryonic growth retardation and increased embryolethality to 37.5% at 10 micrograms/ml (5.0 x 10(-5) M). All embryos in the treated groups exhibited specific morphological defects including hypoplasia of the prosencephalon (forebrain) (100%) and visceral arches (100%). In day 10 embryos cultured for 24 hr, glufosinate significantly reduced the crown-rump length and the number of somite pairs, and produced a high incidence of morphological defects (84.6%) at 10 micrograms/ml. These embryos were characterized by blister in the lateral head (100%), hypoplasia of prosencephalon (57.1%), and cleft lips (42.9%) at 20 micrograms/ml (10.0 x 10(-5) M). Histological examination of the treated embryos showed numerous cell death (pyknotic debris) present throughout the neuroepithelium in the brain vesicle and neural tube, but did not involve the underlying mesenchyme. In micromass culture, glufosinate inhibited the differentiation of midbrain cells in day 12 embryos with 50% inhibition occurring at 0.55 microgram/ml (2.8 x 10(-6) M). The ratios of 50% inhibition concentration for cell proliferation to cell differentiation in limb bud cells were 0.76 and 1.52 in day 11 and 12 embryos, respectively. These findings indicate that glufosinate ammonium is embryotoxic in vitro. In addition to causing growth retardation, glufosinate specifically affected the neuroepithelium of the brain vesicle and neural tube, leading to neuroepithelial cell death.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Developmental+and+dysmorphogenic+effects+of+glufosinate+ammonium+on+mouse+embryos+in+culture

31. Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R., Langeloh, A. (1993): The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicol. Lett. 142, 45-52.

The aim of this study was to assess the teratogenicity of the herbicide glyphosate-Roundup (as commercialized in Brazil) to Wistar rats. Dams were treated orally with water or 500, 750 or 1000 mg/kg glyphosate from day 6 to 15 of pregnancy. Cesarean sections were performed on day 21 of pregnancy, and number of corpora lutea, implantation sites, living and dead fetuses, and resorptions were recorded. Weight and gender of the fetuses were determined, and fetuses were examined for external malformations and skeletal alterations. The organs of the dams were removed and weighed. Results showed a 50%, mortality rate for dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in 15.4, 33.1, 42.0 and 57.3% of fetuses from the control, 500, 750 and 1000 mg/kg glyphosate groups, respectively. We may conclude that glyphosate-Roundup is toxic to the dams and induces developmental retardation of the fetal skeleton.

http://www.ncbi.nlm.nih.gov/pubmed/?term=The+teratogenic+potential+of+the+herbicide+glyphosate-Roundup+in+Wistar+rats.+Toxicol.+Lett.+142%2C+45-52

32. Hietanen, E., Linnainmaa, K., Vainio, H. (1983): Effects of phenoxy herbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Acta Pharma et Toxicol 53, 103112.

The effects of phenoxyacid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), clofibrate, and glyphosate on hepatic and intestinal drug metabolizing enzyme activities were studied in rats intragastrically exposed for 2 weeks. The hepatic ethoxycoumarin O-deethylase activity increased about 2-fold with MCPA. Both 2,4-D and MCPA increased the hepatic epoxide hydrolase activity and decreased the hepatic glutathione S-transferase activity. MCPA also increased the intestinal activities of ethoxycoumarin O-deethylase and epoxide hydrolase. Glyphosate decreased the hepatic level of cytochrome P-450 and monooxygenase activities and the intestinal activity of aryl hydrocarbon hydroxylase. Clofibrate decreased the hepatic activities of UDPglucuronosyltransferase with p-nitrophenol or methylumbelliferone as the substrate. Also 2,4-D decreased the hepatic activity of UDPglucuronosyltransferase with p-nitrophenol as the substrate. MCPA decreased the intestinal activities of UDPglucuronosyltransferase with either p-nitrophenol or methylumbelliferone as the substrate. The results indicate that phenoxyacetic acids, especially MCPA, may have potent effects on the metabolism of xenobiotics. Glyphosate, not chemically related to phenoxyacids, seems to inhibit monooxygenases. Whether these changes are related to the toxicity of these xenobiotics remains to be clarified in further experiments.

http://onlinelibrary.wiley.com/doi/10.1111/j.16000773.1983.tb01876.x/Abstract

33. Fog, L. (2007): Aerial spraying of herbicide “damages DNA”. SciDev.net, May 17, 2007. http://www.scidev.net/en/news/aerial-spraying-of-herbicide-damagesdna.html

Aerial spraying of a herbicide by the Colombian government on the border of Colombia and Ecuador has caused a high degree of DNA damage in local Ecuadorian people, according to a study. The scientists, from the Pontificia Catholic University in Ecuador, analysed blood samples from 24 Ecuadorians living within three kilometres of the border of the two countries. Aerial spraying of a herbicide formulation containing glyphosate sold under the name Roundup by Monsanto — took place on the Colombian side of the border between late 2000 and early 2001. The Colombian government sprays illegal coca plantations used to make cocaine as part of its 'war on drugs'. According to the paper, the application rate of the herbicide (litres per hectare) was 20 times the maximum recommended rate for the formulated product. Half the individuals in the group received spraying directly over their houses, and the blood samples were taken within two months of the spraying taking place.For comparison, blood samples were taken from 21 Ecuadorian individuals living 80 kilometres away from the border, where aerial spraying of the herbicide formulation did not take place. In addition to expected symptoms including vomiting and diarrhoea, blurred vision, and difficulty in breathing the researchers found a significantly higher degree of DNA damage 600 to 800 per cent higher in the people living near the border compared with those 80 kilometres away. The researchers ruled out tobacco, alcohol, non-prescription drugs and asbestos as causing the DNA damage. None of the individuals used or had been exposed to other herbicides or pesticides when the samples were taken. DNA damage may activate genes associated to the development of cancer, lead researcher Cesar Paz y Miño told SciDev.Net, and may also lead to miscarriage or malformations in embryos. Both Colombia and Ecuador have formed national scientific and technical commissions to study the effects of aerially spraying this herbicide formulation, with the Ecuadorian commission concluding it does affect humans and the Colombian commission refuting this claim (see Pesticides used in Colombian war on drugs 'not harmful'). President of the Colombian commission, Alberto Gómez Mejía, told SciDev.Net that it is difficult to establish the real cause of the effects of agrochemicals in humans.

GENETIC ENGINEERING & HEALTH IMPACTS

1. Mesnage R, Clair E, Gress S, Then C, Székács A, Séralini G-E. (2012): Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology. 15 Feb 2012. DOI: 10.1002/jat.2712

The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified Bt insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac Bt toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000 ppm is necrotic and apoptotic from 50 ppm, far below agricultural dilutions (50% lethal concentration 57.5 ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified Bt toxins are not inert on nontarget human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.

2. Qin Shao and Khew-Voon Chin (2011): Survey of American food trends and the growing obesity epidemic. Nutr Res Pract. 5(3): 253-259

The rapid rise in the incidence of obesity has emerged as one of the most pressing global public health issues in recent years. The underlying etiological causes of obesity, whether behavioral, environmental, genetic, or a combination of several of them, have not been completely elucidated. The obesity epidemic has been attributed to the ready availability, abundance, and overconsumption of high-energy content food. We determined here by Pearson's correlation the relationship between food type consumption and rising obesity using the loss-adjusted food availability data from the United States Department of Agriculture (USDA) Economic Research Services (ERS) as well as the obesity prevalence data from the Behavioral Risk Factor Surveillance System (BRFSS) and the National Health and Nutrition Examination Survey (NHANES) at the Centers for Disease Control and Prevention (CDC). Our analysis showed that total calorie intake and consumption of high fructose corn syrup (HFCS) did not correlate with rising obesity trends. Intake of other major food types, including chicken, dairy fats, salad and cooking oils, and cheese also did not correlate with obesity trends. However, our results surprisingly revealed that consumption of corn products correlated with rising obesity and was independent of gender and race/ethnicity among population dynamics in the U.S. Therefore, we were able to demonstrate a novel link between the consumption of corn products and rising obesity trends that has not been previously attributed to the obesity epidemic. This correlation coincides with the introduction of bioengineered corns into the human food chain, thus raising a new hypothesis that should be tested in molecular and animal models of obesity.

3. Daniela Cirnatu, A Jompan, Anca Ileana Sin, Corina Aurelia Zugravu (2011): Multiple organ histopathological changes in broiler chickens fed on genetically modified organism. Rom J Morphol Embryol 52 (1 Suppl): 475-480

Diet can influence the structural characteristics of internal organs. An experiment involving 130 meat broilers was conducted during 42 days (life term for a meat broiler) to study the effect of feed with protein from genetically modified soy. The 1-day-old birds were randomly allocated to five study groups, fed with soy, sunflower, wheat, fish flour, PC starter. In the diet of each group, an amount of protein from soy was replaced with genetically modified soy (I 0%, II 25%, III 50%, IV 75%, V 100% protein from genetically modified soy). The level of protein in soy, either modified, or non-modified, was the same. Organs and carcass weights were measured at about 42 days of age of the birds and histopathology exams were performed during MayJune 2009. No statistically significant differences were observed in mortality, growth performance variables or carcass and organ yields between broilers consuming diets produced with genetically modified soybean fractions and those consuming diets produced with near-isoline control soybean fractions. Inflammatory and degenerative liver lesions, muscle hypertrophy, hemorrhagic necrosis of bursa, kidney focal tubular necrosis, necrosis and superficial ulceration of bowel and pancreatic dystrophies were found in tissues from broilers fed on protein from genetically modified soy. Different types of lesions found in our study might be due to other causes (parasites, viral) superimposed but their presence exclusively in groups fed with modified soy raises some serious questions about the consequences of use of this type of feed.

4. Aris A and Leblanc S. (2011): Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reprod Toxicol, doi:10.1016/j.reprotox.2011.02.004

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

http://www.ncbi.nlm.nih.gov/pubmed/21338670

5. Pusztai A and Bardocz S (2011): Potential health effects of foods derived from genetically modified plants: What are the issues?, TWN Biotechnology & Biosafety Series 14.

In the European Union, the acceptance and regulation of genetically modified (GM) crops/foods is based on the safety data which the biotech companies provide for the European Food Safety Authority (EFSA) and not on the results of EFSA's own investigations. The situation is worse in the USA where there is lax regulation and the commercialisation of GM crops/foods is based on the flawed concept of 'substantial equivalence'. This, without stringent quantitative criteria, can only serve, at best, as an indication of comparability, but at worst, it can be misleading. It is therefore imperative that each GM crop is subjected to, as a minimum, the following:

- ☐ comparison of the composition of the GM and isogenic lines with up-to-date analytical techniques, such as proteomic analysis (2D electrophoresis and mass spectrometric analysis of components).
- ☐ full biochemical, nutritional and toxicological comparison of the in planta expressed transgene product with that of the original gene used for the transformation .
- ☐ microarray analysis of all novel RNA species in the genetically modified plant.
- ☐ molecular examination of possible secondary DNA inserts into the plant genome.
- ☐ full obligatory metabolomic NMR, etc. analysis of the transformed plant.
- ☐ assessment of the variation of known toxins of GM plants grown under different agronomic conditions.
- ☐ determination of the stability to degradation by acid or pepsin or other proteases/hydrolases of GM products, foreign DNA, including the gene construct, promoter, antibiotic resistance marker gene, etc. in the gut of animals in vivo.
- ☐ with GM lectins, including the Bt-toxins, estimation by immunohistology of the presence/absence of epithelial binding in the gut.
- ☐ investigation of the nutritional, immunological, hormonal properties, and allergenicity of GM products using the transgene product isolated from the GM crop and not with recombinant material from E. Coli
- ☐ short- and long-term independent biological risk-assessment tests, first with laboratory animals, followed by human clinical studies of all GM crops/foods themselves and not just the transgene products.

www.twnside.org.sg/title2/biosafety/pdf/bio14.pdf

6. Guimaraes V, Drumare MF, Lereclus D, et al. (2010): In vitro digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity. J Agric Food Chem. 58(5): 3222- 3231

A pepsin resistance test performed at pH 1.2 and with high pepsin to protein ratio is one of the steps of the weight-of-evidence approach used for assessment of allergenicity of new proteins. However, the use of other in vitro digestibility tests, performed in more physiologically relevant conditions and in combination with immunological assays so as to increase the value of the information gained from the studies of stability of a novel protein to digestion for the overall allergenicity assessment, has been proposed. This study then aimed to investigate the stability to digestion of Cry1Ab protoxin and toxin, insecticidal proteins expressed in genetically modified crops, using simulated gastric fluid (SGF) at different pH values and pepsin-to-substrate ratios, in the presence or absence of physiological surfactant phosphatidylcholine (PC). Electrophoresis and immunoblot patterns and residual immunoreactivity of digesta were analyzed. Although Cry1Ab protoxin is extensively degraded at pH 1.2 with high pepsin-to-protein ratio, it is only slightly degraded at pH 2.0 and conserved its immunoreactivity. Furthermore, Cry1Ab proteins were demonstrated to be stable in a more physiologically relevant in vitro digestibility test (pH 2.5, pepsin-to-substrate ratio 1:20 (w/w) with PC). Factors such as pH, SGF composition, and pepsin-to-substrate ratio then greatly influence the digestion of Cry1Ab proteins, confirming that new and more physiologically relevant in vitro digestibility tests should be also considered to study the relationship between the resistance of a protein to digestion and its allergenicity.

7. Tudisco R., Mastellone V., Cutrignelli M.I, Lombardi P, Bovera F., Mirabella N., Piccolo G., Calabro S., Avallone L., Infascelli F. (2010): Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal. The Animal Consortium: 1-10; 4:1662-1671

The presence of DNA fragments in blood and milk from goats fed conventional (control) or Roundup Ready® soybean meal solvent extracted (s.e.; treated) was investigated by using a polymerase chain reaction approach. The same investigation was carried out on blood, skeletal muscle and organs from kids of both groups fed only dams' milk until weaning. Moreover, the possible effects on cell metabolism were evaluated by determination of several specific enzymes in serum, heart, skeletal muscle, liver and kidney. Fragments of the multicopy chloroplast (trnL) gene were found in blood and milk samples from goats of both groups. In kids, the chloroplast fragments were found in samples of both groups. In samples, which proved positive for the presence of chloroplast DNA, fragments of the specific soybean single copy gene (lectin) were detected in several blood and milk samples. The same fragment was also found in control and treated groups of kids. Transgenic fragments were not found in those samples, which were found positive for chloroplast fragments of control groups of either goats or kids. On the contrary, in blood and milk of treated goats, fragments both of the 35S promoter and the CP4 epsps gene were detected. These fragments were also found in treated kids with a significant detection of the 35S promoter in liver, kidney and blood, and of the CP4 epsps gene fragment in liver, kidney, heart and muscle. A significant increase in lactic dehydrogenase, mainly concerning the lactic dehydrogenase-1 isoenzyme was found in heart, skeletal muscle and kidney of treated kids, thus suggesting a change in the local production of the enzyme. Finally, no significant differences were detected concerning kid body and organ weight.

http://www.ncbi.nlm.nih.gov/pubmed/22445119

8. Dona A, Arvanitoyannis IS. (2009): Health risks of genetically modified foods. Crit Rev Food Sci Nutr. 2009; 49(2): 164175

As genetically modified (GM) foods are starting to intrude in our diet concerns have been expressed regarding GM food safety. These concerns as well as the limitations of the procedures followed in the evaluation of their safety are presented. Animal toxicity studies with certain GM foods have shown that they may toxically affect several organs and systems. The review of these studies should not be conducted separately for each GM food, but according to the effects exerted on certain organs it may help us create a better picture of the possible health effects on human beings. The results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects and may alter the hematological, biochemical, and immunologic parameters. However, many years of research with animals and clinical trials are required for this assessment. The use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which may promote cancer.
Jack Heinemann (2009): Report on animals exposed to GM ingredients in animal feed. Prepared for the Commerce Commission of New Zealand.

www.biosafety-info.net/article.php?aid=645

9. de Vendômois, J. S, F. Roullier, D. Cellier, Gilles-Eric Séralini (2009): A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health. Int. Journal of Biological Sciences 5: 706-726.

We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been modified to be tolerant to the broad spectrum herbicide Roundup and thus contains residues of this formulation. MON 810 and MON 863 are engineered to synthesize two different Bt toxins used as insecticides. Approximately 60 different biochemical parameters were classified per organ and measured in serum and urine after 5 and 14 weeks of feeding. GM maize-fed rats were compared first to their respective isogenic or parental non-GM equivalent control groups. This was followed by comparison to six reference groups, which had consumed various other non-GM maize varieties. We applied nonparametric methods, including multiple pairwise comparisons with a False Discovery Rate approach. Principal Component Analysis allowed the investigation of scattering of different factors (sex, weeks of feeding, diet, dose and group). Our analysis clearly reveals for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also noticed in the heart, adrenal glands, spleen and haematopoietic system. We conclude that these data highlight signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM corn. In addition, unintended direct or indirect metabolic consequences of the genetic modification cannot be excluded.

http://www.biolsci.org/v05p0706.htm

10. Brasil FB, Soares LL, Faria TS, Boaventura GT, Sampaio FJ, Ramos CF. (2009): The impact of dietary organic and transgenic soy on the reproductive system of female adult rat. Anat Rec (Hoboken). 292(4): 587594.

The goal of this article was to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile and ovary and uterus morphology. Wistar rats were fed three different diets from weaning until sacrifice at 15 months of age. The three diets were: casein-based diet control group (CG), organic soy-based diet group (OSG), or transgenic soy-based diet group (GMSG). There were no differences in food consumption or in the diet isoflavone components among the groups. Compared with the CG diet, both the OSG and GMSG diets were associated with significant reductions in body weight, serum triglycerides, and cholesterol ($P < 0.05$) (CG = 406 +/- 23.1; 104.3 +/- 13.2; 119.9 +/- 7.3 GMSG = 368 +/- 17.6; 60.3 +/- 4.6; 83.3 +/- 5.7 OSG = 389 +/- 23.5; 72.3 +/- 12.5; 95.5 +/- 8.0, respectively). The volume density of endometrial glandular epithelium was greater in the GMSG group (29.5 +/- 7.17, $P < 0.001$) when compared with the CG (18.5 +/- 7.4) and OSG (20.3 +/- 10.6) groups. The length density of endometrial glandular epithelium was shorter in both GMSG (567.6 +/- 41.1) and OSG (514.8 +/- 144.5) diets compared with the CG ($P < 0.05$) diet. GMSG also resulted in reduced follicle number and increased corpus luteum number compared to the OSG or CG diets ($P < 0.05$). In summary, both GMSG and OSG diets resulted in decreased body weight and lower serum triglyceride and cholesterol levels, and alterations in uterine and ovarian morphology were also observed. The prolonged use of soy-based diets and their relation to reproductive health warrants further investigation.

11. Kilic, A., Akay, M.T. (2008): A three-generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food and Chemical Toxicology 46, 11641170.

For the last ten years, in accordance with the increased use of genetically modified (GM) foods for human and livestock, a large number of feeding studies have been carried out. However, the evidence is still far from proving whether the long-term consumption of GM foods poses a possible danger for human or animal health. Therefore, this study was designed to evaluate the effects of transgenic corn on the rats that were fed through three generations with either GM corn or its conventional counterpart. Tissue samples of stomach, duodenum, liver and kidney were obtained for histopathological examinations. The average diameter of glomeruli, thickness of renal cortex and glomerular volume were calculated and number of affected animals/number of examined animals for liver and kidney histopathology were determined. Amounts of urea, urea nitrogen, creatinine, uric acid, total protein, albumin and globulin were determined; enzyme activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, creatine kinase and amylase were measured in serum samples. No statistically significant differences were found in relative organ weights of rats within groups but there were some minimal histopathological changes in liver and kidney. Changes in creatinine, total protein and globulin levels were also determined in biochemical analysis.

http://www.ncbi.nlm.nih.gov/pubmed/18191319

12. Trabalza-Marinucci M, Brandi G, Rondini C, et al. (2008): A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livestock Science. 113(2): 178190.

This study shows that a diet including insect-resistant Bt176 maize, fed to 53 ewes and their progeny for 3 years, did not have adverse effects on their health or performance and that no horizontal gene transfer to ruminal microorganisms or animal tissues was detected. No differences were observed regarding performance, reproductive traits, haematological parameters, antioxidant defences, lymphocyte proliferative capacity, phagocytosis and intracellular killing of macrophages, and ruminal microbial population characteristics between control and genetically modified (GM) maize-fed animals. Immune response to Salmonella abortus ovis vaccination was more efficient in GM maize fed sheep. No modifications of histological features of tissues were found; however, cytochemical analyses of ruminal epithelium by Ki67 staining provided evidence of proliferative activation of basal cells in all GM maize-fed ewes. Preliminary electron microscopy analyses of the liver and pancreas revealed smaller cell nuclei containing increased amounts of heterochromatin and perichromatin granules in GM maize-fed lambs. Meat protein content and water loss by cooking were slightly affected by the dietary treatment. No transgenic DNA was detected in tissues, blood, and ruminal fluid or ruminal bacteria. Longitudinal studies should be included in evaluation of food safety whenever possible and sheep may be a useful animal model for toxicological assessment.

13. Finamore, A., Roselli, M., Britti, S., Monastra, G., Ambra, R., Turrini, A., Mengheri, E. (2008): Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J. Agric. Food Chem. 56, 1153311539.

This study evaluated the gut and peripheral immune response to genetically modified (GM) maize in mice in vulnerable conditions. Weaning and old mice were fed a diet containing MON810 or its parental control maize or a pellet diet containing a GM-free maize for 30 and 90 days. The immunophenotype of intestinal intraepithelial, spleen, and blood lymphocytes of control maize fed mice was similar to that of pellet fed mice. As compared to control maize, MON810 maize induced alterations in the percentage of T and B cells and of CD4(+), CD8(+), gammadeltaT, and alphabetaT subpopulations of weaning and old mice fed for 30 or 90 days, respectively, at the gut and peripheral sites. An increase of serum IL-6, IL-13, IL-12p70, and MIP-1beta after MON810 feeding was also found. These results suggest the importance of the gut and peripheral immune response to GM crop ingestion as well as the age of the consumer in the GMO safety evaluation.

http://www.ncbi.nlm.nih.gov/pubmed/19007233

14. Velimirov, A., Binter, C., Zentek, J. (2008): Biological effects of transgenic maize NK603 x MON810 fed in long term reproduction studies in mice. Bundesministerium für Gesundheit, amilie und Jugend Report, Forschungsberichte der Sektion IV Band 3/2008, Austria.

The aim of the study was to examine effects of the stacked GM crop NK603 x MON810 in different models of long term feeding studies. So far no negative effects of GM corn varieties have been reported in peer-reviewed publications. But the hypothesis, that effects after long term exposure might become evident in multi-generation studies has rarely been investigated. In this study three designs were used, including a multi-generation study (MGS), a reproductive assessment by continuous breeding (RACB) and a life-term feeding study (LTS), all performed with laboratory mice (strain OF1). The test diets differed only as to the inclusion of 33% NK603 x MON810 corn (GM) versus non-GM corn of a near isogenic line (ISO), both grown under identical conditions in Canada. The MGS also included one group with a non GM corn cultivated in Austria (A REF). All corn varieties used in the MGS and LTS were harvested in 2005, the transgenic and isogenic corn for the RACB were harvested in Canada in 2007. No Austrian corn was used in this case. In the MGS microscopic and ultrastructural investigations were performed to detect changes at the organ and cell level. Gene expression patterns were compared by micro array expression profiles of the intestine as feed-animal interface and by real time PCR. The results of the MGS showed no statistically significant differences concerning parental body mass. The number of females without litters decreased with time in the GM and ISO group, especially in the 4th generation. In the group fed with A REF corn fewer females were without litters, and accordingly more pups were weaned. The production parameters average litter size and weight as well as number of weaned pups were in favour of the ISO group. These differences were also seen in the RACB design and were statistically significant in the 3rd and 4th litters. In addition, the inter-individual variability was higher in the GM group as compared to the other groups. The LTS showed no statistically significant differences in the survival of 3 groups of mice fed the different maize varieties. In the

MGS the continuative investigations revealed differences between the GM and ISO groups. The comparison of organ weights did not indicate directed dietary effects, except for kidneys. The electron histological investigation of the cell nuclei revealed differences as to fibrillar centres, dense fibrillar components and the pore density in hepatocytes, and cells from spleen and pancreas. This could point to an effect of the GM crop on metabolic parameters. Immunohistochemistry revealed no systematic differences in CD3, CD20 positive cells and macrophages in gut tissue. The microarrays showed differences between the feeding groups. When the data of both non-GM feeding groups from MGS were combined and compared to the GM feeding group, the discrimination became more evident. Analyses of metabolic pathways indicated, that the groups differed regarding some important pathways, including interleukin signalling pathway, cholesterol biosynthesis and protein metabolism. Summarizing the findings of this study it can be concluded, that multi-generation studies, especially based on the RACB design are well suited to reveal differences between feeds. The RACB trial showed time related negative reproductive effects of the GM maize under the given experimental conditions. The RACB trial with its specific design with the repeated use of the parental generation is a demanding biological factor for the maternal organism. Compared to the findings in the RACB trials it can be assumed that the physiological stress was considerably lower in the MGS trial. The trial design of using "new" parental generations instead of continuous breeding with the same generation has to be considered as being obviously less demanding. This might have masked the impact of dietary factors on reproductive performance. However, this part of the experiment is valuable as such because it underlines the need for different experimental designs for the assessment of dietary effects that have an unknown impact on animals. The outcome of this study suggests that future studies on the safety of GM feed and food should include reproduction studies. Physiological and genomic traits and depending on the nature of the genetic modification proteomic and metabolomic methods might be taken into consideration as additional tools to the tests performed in this study.

http://www.criigen.org/SiteEn/images/stories/Dossiers/MON810_NK603/nk603-mon810-mice_2008.pdf

15. Malatesta M., Boraldi F., Annovi G., Baldelli B., Battistelli S., Biggiogera M., Quaglino D. (2008): A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. Histochem Cell Biol. 130, 967-77.

Liver represents a suitable model for monitoring the effects of a diet, due to its key role in controlling the whole metabolism. Although no direct evidence has been reported so far that genetically modified (GM) food may affect health, previous studies on hepatocytes from young female mice fed on GM soybean demonstrated nuclear modifications involving transcription and splicing pathways. In this study, the effects of this diet were studied on liver of old female mice in order to elucidate possible interference with ageing. The morpho-functional characteristics of the liver of 24-month-old mice, fed from weaning on control or GM soybean, were investigated by combining a proteomic approach with ultrastructural, morphometrical and immunoelectron microscopical analyses. Several proteins belonging to hepatocyte metabolism, stress response, calcium signalling and mitochondria were differentially expressed in GM-fed mice, indicating a more marked expression of senescence markers in comparison to controls. Moreover, hepatocytes of GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate. This study demonstrates that GM soybean intake can influence some liver features during ageing and, although the mechanisms remain unknown, underlines the importance to investigate the long-term consequences of GM-diets and the potential synergistic effects with ageing, xenobiotics and/or stress conditions.

http://www.ncbi.nlm.nih.gov/pubmed/18648843

16. Séralini, G.-E., Cellier, D., de Vendomois, J.S. (2007): New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch. Environ Contam Toxicol. 52, 596602.

Health risk assessment of genetically modified organisms (GMOs) cultivated for food or feed is under debate throughout the world, and very little data have been published on mid- or long-term toxicological studies with mammals. One of these studies performed under the responsibility of Monsanto Company with a transgenic corn MON863 has been subjected to questions from regulatory reviewers in Europe, where it was finally approved in 2005. This necessitated a new assessment of kidney pathological findings, and the results remained controversial. An Appeal Court action in Germany (Münster) allowed public access in June 2005 to all the crude data from this 90-day rat-feeding study. We independently re-analyzed these data. Appropriate statistics were added, such as a multivariate analysis of the growth curves, and for biochemical parameters comparisons between GMO-treated rats and the controls fed with an equivalent normal diet, and separately with six reference diets with different compositions. We observed that after the consumption of MON863, rats showed slight but dose-related significant variations in growth for both sexes, resulting in 3.3% decrease in weight for males and 3.7% increase for females. Chemistry measurements reveal signs of hepatorenal toxicity, marked also by differential sensitivities in males and females. Triglycerides increased by 24-40% in females (either at week 14, dose 11% or at week 5, dose 33%, respectively); urine phosphorus and sodium excretions diminished in males by 31-35% (week 14, dose 33%) for the most important results significantly linked to the treatment in comparison to seven diets tested. Longer experiments are essential in order to indicate the real nature and extent of the possible pathology; with the present data it cannot be concluded that GM corn MON863 is a safe product.

http://www.ncbi.nlm.nih.gov/pubmed/17356802

17. Domingo, J.L. (2007): Toxicity studies of genetically modified plants: a review of the published literature. Critical Reviews in Food Science and Nutrition, 47:8, 721 733.

According to the information reported by the WHO, the genetically modified (GM) products that are currently on the international market have all passed risk assessments conducted by national authorities. These assessments have not indicated any risk to human health. In spite of this clear statement, it is quite amazing to note that the review articles published in international scientific journals during the current decade did not find, or the number was particularly small, references concerning human and animal toxicological/health risks studies on GM foods. In this paper, the scientific information concerning the potential toxicity of GM/transgenic plants using the Medline database is reviewed. Studies about the safety of the potential use of potatoes, corn, soybeans, rice, cucumber, tomatoes, sweet pepper, peas, and canola plants for food and feed were included. The number of references was surprisingly limited. Moreover, most published studies were not performed by the biotechnology companies that produce these products. This review can be concluded raising the following question: where is the scientific evidence showing that GM plants/food are toxicologically safe?

18. Sharma R, Damgaard D, Alexander T W, Dugan M E R, Aalhus J L, Stanford K and McAllister T A (2006): Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready Canola meal. J. Agric. Food Chem. 54 (5): 1699-1709.

The persistence of plant-derived recombinant DNA in sheep and pigs fed genetically modified (Roundup Ready) canola was assessed by PCR and Southern hybridization analysis of DNA extracted from digesta, gastrointestinal (GI) tract tissues, and visceral organs. Sheep (n = 11) and pigs (n = 36) were fed to slaughter on diets containing 6.5 or 15% Roundup Ready canola. Native plant DNA (high- and low-copy-number gene fragments) and the cp4 epsps transgene that encodes 5-enolpyruvyl shikimate-3-phosphate synthase were tracked in ruminal, abomasal, and large intestinal digesta and in tissue from the esophagus, rumen, abomasum, small and large intestine, liver, and kidney of sheep and in cecal content and tissue from the duodenum, cecum, liver, spleen, and kidney of pigs. High-copy chloroplast-specific DNA (a 520-bp fragment) was detected in all digesta samples, the majority (89-100%) of intestinal tissues, and at least one of each visceral organ sample (frequencies of 3-27%) from sheep and swine. Low-copy rubisco fragments (186- and 540-bp sequences from the small subunit) were present at slightly lower, variable frequencies in digesta (18-82%) and intestinal tissues (9-27% of ovine and 17-25% of porcine samples) and infrequently in visceral organs (1 of 88 ovine samples; 3 of 216 porcine samples). Each of the five cp4 epsps transgene fragments (179-527 bp) surveyed was present in at least 27% of ovine large intestinal content samples (maximum = 64%) and at least 33% of porcine cecal content samples (maximum = 75%). In sheep, transgene fragments were more common in intestinal digesta than in ruminal or abomasal content. Transgene fragments were detected in 0 (esophagus) to 3 (large intestine) GI tract tissues from the 11 sheep and in 0-10 of the duodenal and cecal tissues collected from 36 pigs. The feed-ingested recombinant DNA was not detected in visceral tissues (liver, kidney) of lambs or in the spleen from pigs. Of note, however, one liver and one kidney sample

from the pigs (different animals) were positive for a 278-bp fragment of the transgenic cp4 epsps (denoted F3). Examination of genomic libraries from these tissues yielded no conclusive information regarding integration of the fragment into porcine DNA. This study confirms that feed-ingested DNA fragments (endogenous and transgenic) do survive to the terminal GI tract and that uptake into gut epithelial tissues does occur. A very low frequency of transmittance to visceral tissue was confirmed in pigs, but not in sheep. It is recognized that the low copy number of transgenes in GM feeds is a challenge to their detection in tissues, but there was no evidence to suggest that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material.

http://www.ncbi.nlm.nih.gov/pubmed/16506822

19. Oliveri et al (2006): Temporary depression of transcription in mouse preimplantation embryos from mice fed on genetically modified soybean. 48th Symposium of the Society for Histochemistry. Lake Maggiore (Italy), Sept. 7-10.

20. Agodi A, Barchitta M, Grillo A and Sciacca S (2006): Detection of genetically modified DNA sequences in milk from the Italian market. Int J Hyg Environ Health 209: 81-88.

The possible transfer and accumulation of novel DNA and/or proteins in food for human consumption derived from animals receiving genetically modified (GM) feed is at present the object of scientific dispute. A number of studies failed to identify GM DNA in milk, meat, or eggs derived from livestock receiving GM feed ingredients. The present study was performed in order to: (i) develop a valid protocol by PCR and multicomponent analysis for the detection of specific DNA sequences in milk, focused on GM maize and GM soybean; (ii) assess the stability of transgenic DNA after pasteurization treatment and (iii) determine the presence of GM DNA sequences in milk samples collected from the Italian market. Results from the screening of 60 samples of 12 different milk brands demonstrated the presence of GM maize sequences in 15 (25%) and of GM soybean sequences in 7 samples (11.7%). Our screening methodology shows a very high sensitivity and the use of an automatic identification of the amplified products increases its specificity and reliability. Moreover, we demonstrated that the pasteurization process is not able to degrade the DNA sequences in spiked milk samples. The detection of GM DNA in milk can be interpreted as an indicator of fecal or airborne contamination, respectively, with feed DNA or feed particles, although an alternative source of contamination, possibly recognizable in the natural environment can be suggested. Further studies, performed on a larger number of milk samples, are needed to understand the likely source of contamination of milk collected from the Italian market.

http://europepmc.org/Abstract/MED/16373205

21. Tudisco, R. Lombardi, P. Bovera, F. d'Angelo, D. Cutrignelli, M. I. Mastellone, V., Terzi, V. Avallone, L., Infascelli, F. (2006): Genetically modified soya bean in rabbit feeding: Detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. Animal Science 82, 193199.

The presence of DNA fragments in tissues from rabbits given genetically modified (GM) soya-bean meal (solvent extracted) was investigated by using the polymerase chain reaction (PCR) approach. Moreover, the possible effects on cell metabolism were evaluated by determination of several specific enzymes in serum, heart, skeletal muscle, liver and kidney. The chloroplast sequence for tRNA Leu by using the Clor1/Clor2 primers designed on chloroplast trnL sequence was clearly detected. On the contrary, two couples of species specific primers for conventional (Le1-5/Le 1-3 which amplifies the soya bean lectin gene) and genetically modified (35S1/35S2 which amplifies the 35S CMV promoter that is present in the genomic structure of GM soya bean) soya bean were not found in all samples. No differences in enzyme levels were detected in serum, but a significant increase of lactic dehydrogenase, mainly concerning the LDH1 isoenzyme was found in particular in kidney and heart but not in the muscle, thus suggesting a potential alteration in the local production of the enzyme. Finally, no significant differences were detected concerning body weight, fresh organ weights and no sexual differences were detected.

http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=778124

22. Yum HY, Lee SY, Lee KE, Sohn MH, Kim KE. (2005): Genetically modified and wild soybeans: an immunologic comparison. Allergy Asthma Proc. May-Jun 2005; 26(3): 210-216.

Most traits introduced into genetically engineered crops result from the expression of new proteins. As the first step toward assessing the allergenic potential of genetically modified organism (GMO) food, immunologic and physicochemical characterizations are needed. We prepared crude extract from GMO soybeans, wild soybeans, curd, and soy milk and then performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After acidification with HCl, the samples were separated to globulin and whey. To evaluate changes in protein composition, either the samples were heated or pepsin was added. Polymerase chain reaction with primer encoding the 35S-promotor and the 3-enol-pyruvyl-shikimat-5-phosphat-synthase gene were performed, respectively, to detect the GMO component. SDS-PAGE results showed definite protein bands at 80 kDa in GMO soybean, 50 kDa in wild soybean, and a similar distribution of protein bands was noticed below 40 kDa. It was difficult to observe protein distribution because of modifications that occurred during processing in soybean-processed products. After heating, proteins of GMO and wild soybeans showed similar distributions and no distinct bands were detected at 50 and 80 kDa. Although SDS-PAGE analyses of raw GMO and wild soybeans differed, the same protein bands of 68, 37, and 20 kDa were observed in the globulin fraction after acidification. After adding pepsin, 20- and 68-kDa bands were found preserved in GMO and wild soybeans. The polymerase chain reaction procedures with primers specific to GMO soybeans showed that GMO soybeans and some curd samples included a GMO component. The skin test results of 49 patients showed 13 positive results to wild soybeans and 8 positive results to GMO soybeans. One patient had a positive skin test result to GMO soybeans only. Sera from nine patients with positive skin tests to the crude extract and a positive capsulated allergen product test to the soybean antigen were used for the immunoblotting of GMO and wild soybeans. GMO soybeans revealed

a unique strong immunoglobulin E binding band at 25 kDa in some patients and wild soybeans showed a strong immunoglobulin E binding band at 30-36 kDa. To assess the allergenicity of GMO food, more research, including a selection of controlled sample materials and immunoassays of qualified sera, is needed.

23. Heritage, John (2004): The fate of transgenes in the human gut. Heritage J. Nat Biotech., 2: 170-172

In its 2003 GM Science Review, the UK government concluded that trans-kingdom transfer of DNA from GM plants to bacteria is "unlikely to occur because of a series of well-established barriers" and illustrated support for this position from experimental evidence in peer-reviewed literature... Much of this work has been done using animal studies, and very little is known about the process in humans. The data presented in the paper [Netherwood et al, showing gut microbes may acquire and harbour genes from genetically modified plants] support the conclusion that gene flow from transgenic plants to the gut microflora does occur. Furthermore, because transfer events seem to have occurred in three of the seven subjects examined, it may be that trans-kingdom gene transfers are not as rare as suggested by the UK GM Science Review Panel.

24. Netherwood T., Martín-Orúe S.M., O'Donnell A.G., Gockling S., Graham J., Mathers J.C., Gilbert H.J. (2004): Assessing the survival of transgenic plant DNA in the human gastro-intestinal tract. Nature Biotechnology 22, 204209

The inclusion of genetically modified (GM) plants in the human diet has raised concerns about the possible transfer of transgenes from GM plants to intestinal microflora and enterocytes. The persistence in the human gut of DNA from dietary GM plants is unknown. Here we study the survival of the transgene epsps from GM soya in the small intestine of human ileostomists (i.e., individuals in which the terminal ileum is resected and digesta are diverted from the body via a stoma to a colostomy bag). The amount of transgene that survived passage through the small bowel varied among individuals, with a maximum of 3.7% recovered at the stoma of one individual. The transgene did not survive passage through the intact gastrointestinal tract of human subjects fed GM soya. Three of seven ileostomists showed evidence of low-frequency gene transfer from GM soya to the microflora of the small bowel before their involvement in these experiments. As this low level of epsps in the intestinal microflora did not increase after consumption of the meal containing GM soya, we conclude that gene transfer did not occur during the feeding experiment.

http://www.ncbi.nlm.nih.gov/pubmed/14730317

25. Vecchio L., Cisterna B., Malatesta M., Martin T.E., Biggiogera M. (2004): Ultrastructural analysis of testes from mice fed on genetically modified soybean. Eur J. Histochem. 48: 448 54.

We have considered the possible effects of a diet containing genetically modified (GM) soybean on mouse testis. This organ, in fact, is a well known bioindicator and it has already been utilized, for instance, to monitor pollution by heavy metals. In this preliminary study, we have focussed our attention on Sertoli cells, spermatogonia and spermatocytes by means of immunoelectron microscopy. Our results point out that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II is decreased in 2 and 5 month-old GM-fed mice, and is restored to normal at 8 months. In GM-fed mice of all ages considered, the number of perichromatin granules is higher and the nuclear pore density lower. Moreover, we found enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells. A possible role played by traces of the herbicide to which the soybean is resistant is discussed.

http://www.ncbi.nlm.nih.gov/pubmed/15718213

26. Müller, W. (2004): Recherche und Analyse bezuglich humantoxikologischer Risiken von gentechnisch veränderten Soja- und Maispflanzen. Eco-risk (Buro fur Ökologische Risikoforschung), Vienna, April 10

All approved genetically modified organisms (GMOs) are regarded as safe for daily human consumption. On the other hand, reports on possible hazards of GMOs have been circulated through various media. This study reviews the scientific literature on the health hazards of the Roundup Ready soybean and Bt-Maize crops. The toxicological assessment of the application for authorisation of Roundup Ready soybean was analyzed. In the case of Bt-maize a thorough investigation of the application for authorisation has recently been carried out, by an Austrian research group, and is therefore not included in this study. However, some of the latest food conversion studies of Bt-maize has been investigated. The data shows that valid information on the chronic toxicity potential of Roundup Ready soybeans and Btmaize varieties is still missing. The main focus in the risk assessment of GMOs is based on the principle of substantial equivalence. This principle is an unproven hypothesis which infers human health hazards (including chronic hazards) from a comparison of ingredients between the genetically modified variety and the conventional counterpart. If no differences are detected the GMO is regarded as safe on the basis of this unproven hypothesis. In general, there is no consistency in the methods and the interpretations when substantial equivalence is claimed. Independent and long term research with rats, which has been undertaken after the approval of the Roundup Ready soybean and studies with Bt-maize varieties, shows that potential hazards may have been overlooked due to insufficient and short term test methods before the approval process. Several reports on the human health hazards of Roundup Ready soybeans, such as a rise of allergies, have been investigated. No published scientific study has been found, that supports or rejects this "anecdotal evidence". It is suggested that the risk assessment of GMO should be based on regular chronic toxicity testing, using the whole plant. Furthermore, an immediate re-evaluation of already approved GMOs is highly recommended. Independent risk research is needed in order to address these safety questions, which are currently not addressed during the authorisation process of GMOs.

www.keinegentechnik.de/bibliothek/verbraucher/studien/ecoris_analyse_humantoxikologie_040410.pdf

27. Malatesta M., Biggiogera M., Manuali E., Rocchi M.B., Baldelli B., Gazzanelli G. (2003): Fine structural analysis of pancreatic acinar cell nuclei from mice fed on GM soybean. Eur J Histochem. 47, 3858.

We carried out ultrastructural morphometrical and immunocytochemical analyses on pancreatic acinar cell nuclei from mice fed on genetically modified (GM) soybean, in order to investigate possible structural and molecular modifications of nucleoplasmic and nucleolar constituents. We found a significant lowering of nucleoplasmic and nucleolar splicing factors as well as a perichromatin granule accumulation in GM-fed mice, suggestive of reduced post-transcriptional hnRNA processing and/or nuclear export. This is in accordance to already described zymogen synthesis and processing modifications in the same animals.

http://www.ncbi.nlm.nih.gov/pubmed/14706936

28. Bernstein JA, Bernstein IL, Bucchini L, et al. (2003): Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect. 111(8): 1114-1121.

Technology has improved the food supply since the first cultivation of crops. Genetic engineering facilitates the transfer of genes among organisms. Generally, only minute amounts of a specific protein need to be expressed to obtain the desired trait. Food allergy affects only individuals with an abnormal immunologic response to food--6% of children and 1.5-2% of adults in the United States. Not all diseases caused by food allergy are mediated by IgE. A number of expert committees have advised the U.S. government and international organizations on risk assessment for allergenicity of food proteins. These committees have created decision trees largely based on assessment of IgE-mediated food allergenicity. Difficulties include the limited availability of allergen-specific IgE antisera from allergic persons as validated source material, the utility of specific IgE assays, limited characterization of food proteins, cross-reactivity between food and other allergens, and modifications of food proteins by processing. StarLink was a corn variety modified to produce a Bacillus thuringiensis (Bt) endotoxin, Cry9C. The Centers for Disease Control and Prevention investigated 51 reports of possible adverse reactions to corn that occurred after the announcement that StarLink, allowed for animal feed, was found in the human food supply. Allergic reactions were not confirmed, but tools for post-market assessment were limited. Workers in agricultural and food preparation facilities have potential inhalation exposure to plant dusts and flours. In 1999, researchers found that migrant health workers can become sensitized to certain Bt spore extracts after exposure to Bt spraying.

29. Pryme IF, Lembcke R (2003): In vivo studies on possible health consequences of genetically modified food and feed with particular regard to ingredients consisting of genetically modified plant materials. Nutr Health 17: 1-8.

This synopsis reviews published in vivo studies on possible health consequences of genetically modified food and feed where the ingredients in question have consisted of genetically modified plant materials. The following, however, have not been taken into consideration:--ingredients consisting of genetically modified microorganisms or parts of animals/fish--ingredients produced by/from genetically modified organisms but without any DNA present--studies on consequences for the environment or biodiversity--in vitro studies or computer simulations. According to a Norwegian report "Gen-mat" (NOU 2000:29), and a more recent search in Medline and Citations Index, to our knowledge a total of ten studies have been published on the health effects of GM-foods and feeds. In this minireview the data made available in these published studies is discussed.

http://www.ncbi.nlm.nih.gov/pubmed/12803276

30. Martín-Orúe S.M., O'Donnell A.G., Ariño J., Netherwood T., Gilbert H.J., Mathers J.C. (2002): Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. British Journal of Nutrition 87, 533542.

The inclusion of genetically modified (GM) foods in the human diet has caused considerable debate. There is concern that the transfer of plant-derived transgenes to the resident intestinal microflora could have safety implications. For these gene transfer events to occur, the nucleic acid would need to survive passage through the gastrointestinal tract. The aim of the present study was to evaluate the rate at which transgenes, contained within GM soya and maize, are degraded in gastric and small bowel simulations. The data showed that 80 % of the transgene in naked GM soya DNA was degraded in the gastric simulations, while no degradation of the transgenes contained within GM soya and maize were observed in these acidic conditions. In the small intestinal simulations, transgenes in naked soya DNA were degraded at a similar rate to the material in the soya protein. After incubation for 30 min, the transgenes remaining in soya protein and naked DNA were 52 (SEM 131) % AND 34 (sem 175) %, respectively, and at the completion of the experiment (3 h) these values were 5 % and 3 %, respectively. In contrast to the soya transgene, the maize nucleic acid was hydrolysed in the small intestinal simulations in a biphasic process in which approximately 85 % was rapidly degraded, while the rest of the DNA was cleaved at a rate similar to that in the soya material. Guar gum and tannic acid, molecules that are known to inhibit digestive enzymes, did not influence the rate of transgene degradation in soya protein. In contrast guar gum reduced the rate of transgene degradation in naked soya DNA in the initial stages, but the polysaccharide did not influence the amount of nucleic acid remaining at the end of the experiment. Tannic acid reduced the rate of DNA degradation throughout the small bowel simulations, with 21 (sem 54) % and 2 (sem 18) % of the naked soya DNA remaining in the presence and absence of the phenolic acid, respectively. These data indicate that some transgenes in GM foods may survive passage through the small intestine.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Degradation+of+transgenic+DNA+from+genetically+modified+soya+and+maize+in+human+intestinal+simulations.+British+Journal+of+Nutrition+87%2C+533%E2%80%93542.

31. Schubert, D. (2002): A different perspective on GM food. Nature Biotechnology 20, 969.

In particular, I believe that insufficient attention has been paid to three important issues: first, introduction of the same gene into two different types of cells can produce two very distinct protein molecules; second, the introduction of any gene, whether from a different or the same species, usually significantly changes overall gene expression and therefore the phenotype of the recipient cell; and third, enzymatic pathways introduced to synthesize small molecules, such as vitamins, could interact with endogenous pathways to produce novel molecules. The potential consequence of all of these perturbations could be the biosynthesis of molecules that are toxic, allergenic, or carcinogenic. And there is no a priori way of predicting the outcome. In what follows I outline these concerns and argue that GM food is not a safe option, given our current lack of understanding of the consequences of recombinant technology. The biological activity of a protein can be modified by gene splicing, which alters the primary amino acid sequence, and by the post-translational attachment of such moieties as phosphate, sulfate, sugars, or lipids. The nature of these modifications is markedly dependent upon the cell type in which the protein is expressed. With our current state of knowledge, however, there is no way of predicting either the modifications or their biological effects. Therefore, a toxin that is harmless to humans when made in bacteria could be modified by plant cells in many ways, some of which might be harmful. The second concern is the potential for the introduction of a foreign gene to either evoke the synthesis of toxic, carcinogenic, teratogenic, or allergenic compounds, or down regulate the synthesis of a beneficial plant molecule. Introduction of one gene usually alters the gene expression pattern of the whole cell, and typically each cell type of the organism will respond differently. Although these sorts of unpredicted changes in gene expression and function are frequently observed, they have received very little attention. Furthermore, they are not unexpected. The maintenance of a specific cell phenotype involves a very precise balancing act of gene regulation, and any perturbation might be

expected to change the overall patterns of gene expression. The problem, as with secondary modifications, is that there is currently no way to predict the resultant changes in protein synthesis. Third, the introduction of genes for all or part of a new enzymatic pathway into plants could lead to the synthesis of unexpected or even totally novel products through an interaction with endogenous pathways. Some of these products could be toxic. A worst-case scenario would be that an introduced bacterial toxin is modified to make it toxic to humans. Prompt toxicity might be rapidly detected once the product entered the marketplace if it caused a unique disease, and if the food were labeled for traceability, as were the GM batches of tryptophan. However, cancer or other common diseases with delayed onset would take decades to detect, and might never be traced to their cause.

32. Malatesta M., Caporaloni C., Gavaudan S., Rocchi M.B., Serafini S., Tiberi C., Gazzanelli G. (2002): Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct Funct. 27, 173180.

No direct evidence that genetically modified (GM) food may represent a possible danger for health has been reported so far; however, the scientific literature in this field is still quite poor. Therefore, we carried out an ultrastructural morphometrical and immunocytochemical study on hepatocytes from mice fed on GM soybean, in order to investigate eventual modifications of nuclear components of these cells involved in multiple metabolic pathways related to food processing. Our observations demonstrate significant modifications of some nuclear features in GM-fed mice. In particular, GM fed-mice show irregularly shaped nuclei, which generally represents an index of high metabolic rate, and a higher number of nuclear pores, suggestive of intense molecular trafficking. Moreover, the roundish nucleoli of control animals change in more irregular nucleoli with numerous small fibrillar centres and abundant dense fibrillar component in GM-fed mice, modifications typical of increased metabolic rate. Accordingly, nucleoplasmic (snRNPs and SC-35) and nucleolar (fibrillarin) splicing factors are more abundant in hepatocyte nuclei of GM-fed than in control mice. In conclusion, our data suggest that GM soybean intake can influence hepatocyte nuclear features in young and adult mice; however, the mechanisms responsible for such alterations remain unknown

http://www.ncbi.nlm.nih.gov/pubmed/12441651

33. Ewen SW, Pusztai A (1999): Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet 354: 1353-4

Diets containing genetically modified (GM) potatoes expressing the lectin Galanthus nivalis agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

http://www.ncbi.nlm.nih.gov/pubmed/10533866

34. Schubbert R, Hohlweg U, Renz D and Doerfler W (1998): On the fate of orally ingested foreign DNA in mice: chromosal association and placental transmission to the fetus. Molecular Genomics and Genetics, 259 (6): 569-76.

We have previously shown that, when administered orally to mice, bacteriophage M13 DNA, as a paradigm foreign DNA without homology to the mouse genome, can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall, and reach the nuclei of leukocytes, spleen and liver cells. Similar results were obtained when a plasmid containing the gene for the green fluorescent protein (pEGFP-C1) was fed to mice. In spleen, the foreign DNA was detected in covalent linkage to DNA with a high degree of homology to mouse genes, perhaps pseudogenes, or to authentic E. coli DNA. We have now extended these studies to the offspring of mice that were fed regularly during pregnancy with a daily dose of 50 microg of M13 or pEGFP-C1 DNA. Using the polymerase chain reaction (PCR) or the fluorescent in situ hybridization (FISH) method, foreign DNA, orally ingested by pregnant mice, can be discovered in various organs of fetuses and of newborn animals. The M13 DNA fragments have a length of about 830 bp. In various organs of the mouse fetus, clusters of cells contain foreign DNA as revealed by FISH. The foreign DNA is invariably located in the nuclei. We have never found all cells of the fetus to be transgenic for the foreign DNA. This distribution pattern argues for a transplacental pathway rather than for germline transmission which might be expected only after long-time feeding regimens. In rare cells of three different fetuses, whose mothers have been fed with M 13 DNA during gestation, the foreign DNA was detected by FISH in association with both chromatids. Is maternally ingested foreign DNA a potential mutagen for the developing fetus?

http://www.ncbi.nlm.nih.gov/pubmed/9819049

35. Fares N H, El-Sayed A K (1998): Fine structural changes in the ileum of mice fed on endotoxin treated potatoes and transgenic potatoes. Natural Toxins 6 (6): 219-33.

The present work has been designed to study the effect of feeding on transgenic potatoes, which carry the CryI gene of Bacillus thuringiensis var. kurstaki strain HD1, on the light and electron microscopic structure of the mice ileum, in comparison with feeding on potatoes treated with the 'delta-endotoxin' isolated from the same bacterial strain. The microscopic architecture of the enterocytes of the ileum of both groups of mice revealed certain common features such as the appearance of mitochondria with signs of degeneration and disrupted short microvilli at the luminal surface. However, in the group of mice fed on the 'delta-endotoxin', several villi appeared with an abnormally large number of enterocytes (151.8 in control group versus 197 and 155.8 in endotoxin and transgenic-treated groups, respectively). Fifty percent of these cells were hypertrophied and multinucleated. The mean area of enterocyte was significantly increased (105.3 microm(2) in control group versus 165.4 microm(2) and 116.5 microm(2) in endotoxin and transgenic-treated groups, respectively). Several forms of secondary lysosomes or auotophagic vacuoles were recognized in these cells. These changes were confirmed with the scanning electron microscope which revealed a remarkable increase in the topographic contour of enterocytes (23 microm in control group versus 44 microm and 28 microm in endotoxin and transgenic-treated groups, respectively) at the divulged surface of the villi. The basal lamina along the base of the enterocytes was damaged at several foci. Several disrupted microvilli appeared in association with variable-shaped cytoplasmic fragments. Some of these fragments contained endoplasmic reticulum, as well as ring-shaped annulate lamellae. In addition, the Paneth cells were highly activated and contained a large number of secretory granules. These changes may suggest that delta-endotoxin-treated potatoes resulted in the development of hyperplastic cells in the mice ileum. Although mild changes are reported in the structural configuration of the ileum of mice fed on transgenic potatoes, nevertheless, thorough tests of these new types of genetically engineered crops must be made to avoid the risks before marketing.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Fine+structural+changes+in+the+ileum+of+mice+fed+on+endotoxin+treated+potatoes+and+transgenic+potatoes.+Natural+Toxins+6+%286%29%3A+219-33.#

36. Nordlee J A, Taylor S L, Townsend JA, Thomas LA, Bush RK (1996): Identification of a Brazil-Nut allergen in transgenic soybeans, N Engl J Med 1996, 334: 668-92.

On radioallergosorbent testing of pooled serum from four subjects allergic to Brazil nuts, protein extracts of transgenic soybean inhibited binding of IgE to Brazil-nut proteins. On immunoblotting, serum IgE from eight of nine subjects bound to purified 2S albumin from the Brazil nut and the transgenic soybean. On skin-prick testing, three subjects had positive reactions to extracts of Brazil nut and transgenic soybean and negative reactions to soybean extract. 2S albumin is probably a major Brazil-nut allergen, and the transgenic soybeans analyzed in this study contain this protein. Our study shows that an allergen from a food known to be allergenic can be transferred into another food by genetic engineering.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Identification+of%20a%20Brazil-Nut%20allergen%20in%20transgenic%20soybeans%2C%20N%20Engl%20J%20Med%201996%2C%20334%3A%20668-92.

37. Mayeno AN, Gleich GJ. Eosinophilia-myalgia syndrome and tryptophan production (1994): A cautionary tale. Trends Biotechnol. Sep 1994; 12(9): 346-352.

An epidemic of a new disease, termed eosinophilia-myalgia syndrome, occurred in the USA in 1989. This syndrome was linked to the consumption of l-tryptophan manufactured by a single company utilizing a fermentation process. All the findings indicate that the illness was probably triggered by an impurity formed when the manufacturing conditions were modified. This outbreak highlights the need for close monitoring of the chemical purity of biotechnology-derived products, and for rigorous testing of such products following any significant changes to the manufacturing process.

38. Hines FA. Memorandum to Linda Kahl on the Flavr Savr tomato (1993): (Pathology Review PR152; FDA Number FMF000526): Pathology Branch's evaluation of rats with stomach lesions from three four-week oral (gavage) toxicity studies (IRDC Study Nos. 677002, 677004, and 677005) and an Expert Panel's report. US Department of Health & Human Services. 16 June 1993.

http://www.biointegrity.org/FDAdocs/17/view1.html

BACKGROUND INFORMATION

The Flavr Savr tomato was developed through transgenic techniques. A "wholesomeness" study was undertaken by the sponsor (Calgene, Inc.) to ensure that this food possesses no unexpected toxicity. Three sequential 28-day studies were done at the International Research Development Corporation (IRDC), Mattawan, Michigan, in which groups of male and female rats were fed via gavge either a transgenic tomato, a non-transgenic tomato, or distilled water. In the first study, no gross or microscopic lesions were reported in the stomach of any rat. The second study included two lines of transgenic tomato that were distinct from the transgenic line that was the subject of the first study. In the second study, gross lesions were described in the stomachs of four out of twenty female rats fed one of the two lines of transgenic tomato. The lesions were reported histologically as gastric necrosis and later identified as gastric erosions, i.e., the two terms are synonymous under the conditions of this study since "necrotic" or dead cells occured in the superficial mucosa of the stomach. This lesion, gastric erosion, was not reported by IRDC in any other animals from the second study. The IRDC report stated: "[t]he CR3-623 transgenic tomato dosed to females did suggest a possible treatment-related mild, focal necrosis of the galndular stomach in 4 of 20 animals". The third study was described as a "repeat" study and included the single transgenic line that had four of twenty female rats with stomach erosions in the second study, as well as the control line from which that transgenic line originated. In the third study, the tomatoes used were frozen or frozen-lyophilized (concentrated) the contrast to the first two studies in which fresh tomatoes were used. Gross and microscopic gastric erosions were seen in male and female control rats dosed with dionized water, in male and female rats fed the non-transgenic tomato, and in female rats fed the transgenic tomato. The Sponsor's IRDC report concluded that the "[h]istomorphology and the pattern of incidence of the necrosis and erosion suggested that these lesions were incidental in nature and unrelated to the respective test articles". An "Expert Panel" was organized by Environ at the request of Calgene, Inc. and according to the Expert Panel Report, they were assembled to review the "data relating to the demonstration of the safety of the FLAVR SAVR tomato". According to the Expert Panel Report, an independent pathology review of the stomach slides "to evaluate the incidence and significance of the observed stomach lesions" was conducted by Pathco, Inc., and a report (the PWG report) was provided to the "Expert Panel". The pathology Branch (PB), Division of General Science Support, Office of Scientific Analysis and Support, Center for Food Safety and Applied Nutrition, Food and Drug Administration was requested to review the stomach data and to indicate whether the PB had any concerns about the stomach lesions.

CONCLUSION: There is considerable disparity in the reported findings of gastric erosions or necrosis lesions from the three studies reported by Calgene, Inc. This disparity has not been adequately addressed or explained by the sponsor or the laboratory (IRDC) where the study was conducted. The Expert Panel report and the PWG report also did not address or explain this disparity. The criteria for qualifying a lesion as incidental were not provided in the Sponsor's report. Without explanation or other information which was lacking in the submitted data as mentioned in the attached pathology report (the Pathology Branch's Pathology Review PR-152), the Pathology Branch is unable to determine whether or not the gastric erosions or necrosis in these studies are "incidental" findings as reported by the Sponsor.

CHANGES IN NUTRITIONAL COMPOSITION

1. Zobiole L.H.S., Oliveira R.S., Visentainer J.V., Kremer R.J., Bellaloui N., Yamada T. (2010): Glyphosate affects seed composition in glyphosate-resistant soybean. J. Agric. Food Chem. 58 (7), 45174522.

The cultivation of glyphosate-resistant (GR) soybeans has continuously increased worldwide in recent years mainly due to the importance of glyphosate in current weed management systems. However, not much has been done to understand eventual effects of glyphosate application on GR soybean physiology, especially those related to seed composition with potential effects on human health. Two experiments were conducted to evaluate the effects of glyphosate application on GR soybeans compared with its near-isogenic non-GR parental lines. Results of the first experiment showed that glyphosate application resulted in significant decreases in shoot nutrient concentrations, photosynthetic parameters, and biomass production. Similar trends were observed for the second experiment, although glyphosate application significantly altered seed nutrient concentrations and polyunsaturated fatty acid percentages. Glyphosate resulted in significant decreases in polyunsaturated linoleic acid (18:2n-6) (2.3% decrease) and linolenic acid (18:3n-3) (9.6% decrease) and a significant increase in monounsaturated fatty acids 17:1n-7 (30.3% increase) and 18:1n-7 (25% increase). The combined observations of decreased photosynthetic parameters and low nutrient availability in glyphosate-treated plants may explain potential adverse effects of glyphosate in GR soybeans.

http://www.ncbi.nlm.nih.gov/pubmed/20307082

2. Lappe M.A., Bailey E.B., Childress C., Setchell K.D.R. (1999): Alterations in clinically important phytoestrogens in genetically modified, herbicide-tolerant soybeans. J Med Food, 1, 241245.

The growing clinical interest in and use of soybean-based food products or extracts to increase dietary phytoestrogen intake makes the precise composition of the key biologically active ingredients of soybeans, notably genistin and daidzin, of substantial medical interest. Conventional soybeans are increasingly being replaced by genetically modified varieties. We analyzed the phytoestrogen concentrations in two varieties of genetically modified, herbicide-tolerant soybeans and their isogenic conventional counterparts grown under similar conditions. An overall reduction in phytoestrogen levels of 12-14% was observed in the genetically altered soybean strains, mostly attributable to reductions in the concentrations of genistin and, to a lesser extent, in daidzin. Significant sample-to-sample variability in these two phytoestrogens, but not in glycitin, was evident in the genetically altered soybeans. Given the high biological potency of isoflavones and their metabolic conversion products, these data suggest that genetically modified soybeans may be less potent sources of clinically relevant phytoestrogens than their conventional precursors. These observations, if confirmed in other soybean varieties, heighten the importance of establishing baselines of expected isoflavone levels in transgenic and conventional soy products to ensure uniformity of clinical results. Disclosure of the origins and isoflavone composition of soyfood products would be a valuable adjunct to clinical decision-making.

http://online.liebertpub.com/doi/abs/10.1089/jmf.1998.1.241

3. Shewmaker CK, Sheehy JA, Daley M, Colburn S and Ke DY (1999): Seed-specific over-expression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant J, 20 (4): 401-412X.

A bacterial phytoene synthase (crtB) gene was overexpressed in a seed-specific manner and the protein product targeted to the plastid in Brassica napus (canola). The resultant embryos from these transgenic plants were visibly orange and the mature seed contained up to a 50-fold increase in carotenoids. The predominant carotenoids accumulating in the seeds of the transgenic plants were alpha and beta-carotene. Other precursors such as phytoene were also detected. Lutein, the predominant carotenoid in control seeds, was not substantially increased in the transgenics. The total amount of carotenoids in these seeds is now equivalent to or greater than those seen in the mesocarp of oil palm. Other metabolites in the isoprenoid pathway were examined in these seeds. Sterol levels remained essentially the same, while tocopherol levels decreased significantly as compared to non-transgenic controls. Chlorophyll levels were also reduced in developing transgenic seed. Additionally, the fatty acyl composition was altered with the transgenic seeds having a relatively higher percentage of the 18 : 1 (oleic acid) component and a decreased percentage of the 18 : 2 (linoleic acid) and 18 : 3 (linolenic acid) components. This dramatic increase in flux through the carotenoid pathway and the other metabolic effects are discussed.

http://www.ncbi.nlm.nih.gov/pubmed/10607293

ENVIRONMENTAL IMPACTS

1. Niels Holst, Andreas Lang, Gabor Lövei, Mathias Otto (2013): Increased mortality is predicted of Inachis io larvae caused by Bt-maize pollen in European farmland. Ecological Modelling. 250: 126133

A potential environmental risk of the field cultivation of insect-resistant (Bt-toxin expressing) transgenic maize (Zea mays) is the consumption of Bt-containing pollen by herbivorous larvae of butterflies (Lepidoptera). Maize is wind-pollinated, and at flowering time large amounts of pollen can be deposited on various plants growing in the landscape, leading to inadvertent ingestion of toxic pollen with plant biomass consumed by these butterfly larvae. To examine the possible effect of this coincidence, we focused our study on the protected butterfly Inachis io and two regions of Europe. Using climatic records, maize and butterfly phenology data, we built a simulation model of the butterfly's annual life cycle, over-laid with the phenology of maize pollen deposition on the leaves of the food plant Urtica dioica, and linked these with the doseresponse curve of I. io larvae to Bt-maize pollen (event MON810). The simulations indicated that in Northern Europe, where I. io is univoltine, Bt-maize pollen would not be present on the food plant at the same time as the I. io larvae. However, in Central and Southern Europe, where I. io is bivoltine, Bt-maize pollen and the second generation I. io larvae would coincide, and an increased mortality of the larvae was predicted. This prediction differs from earlier studies which predicted negligible effect of field-grown Bt-maize on I. io larvae. Our model is an improvement over previous efforts since it is based on more detailed, empirical data, includes more biological detail, and provides explicit estimation of all model parameters. The model is open-source software and is available for re-use and for modelling the effects on other species or regions.

http://www.sciencedirect.com/science/article/pii/S0304380012005315

2. John M Pleasants and Karen S Oberhauser (2013): Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity. Vol. 6(2): 135144

The size of the Mexican overwintering population of monarch butterflies has decreased over the last decade. Approximately half of these butterflies come from the U.S. Midwest where larvae feed on common milkweed. There has been a large decline in milkweed in agricultural fields in the Midwest over the last decade. This loss is coincident with the increased use of glyphosate herbicide in conjunction with increased planting of genetically modified (GM) glyphosate-tolerant corn (maize) and soybeans (soya). We investigate whether the decline in the size of the overwintering population can be attributed to a decline in monarch production owing to a loss of milkweeds in agricultural fields in the Midwest. We estimate Midwest annual monarch production using data on the number of monarch eggs per milkweed plant for milkweeds in different habitats, the density of milkweeds in different habitats, and the area occupied by those habitats on the landscape. We estimate that there has been a 58% decline in milkweeds on the Midwest landscape and an 81% decline in monarch production in the Midwest from 1999 to 2010. Monarch production in the Midwest each year was positively correlated with the size of the subsequent overwintering population in Mexico. Taken together, these results strongly suggest that a loss of agricultural milkweeds is a major contributor to the decline in the monarch population. The smaller monarch population size that has become the norm will make the species more vulnerable to other conservation threats.

3. Lincoln P Brower, Orley R Taylor, Ernest H Williams, Daniel A Slayback, Raul R Zubieta and M Isabel Ramirez (2012): Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity. Vol. 5(2): 95-100

During the 20092010 overwintering season and following a 15-year downward trend, the total area in Mexico occupied by the eastern North American population of overwintering monarch butterflies reached an all-time low. Despite an increase, it remained low in 20102011.Although the data set is small, the decline in abundance is statistically significant using both linear and exponential regression models.Three factors appear to have contributed to reduce monarch abundance: degradation of the forest in the overwintering areas; the loss of breeding habitat in the United States due to the expansion of GM herbicide-resistant crops, with consequent loss of milkweed host plants, as well as continued land development; and severe weather.This decline calls into question the long-term survival of the monarchs' migratory phenomenon.

4. Hilbeck A, McMillan JM, Meier M, Humbel A, Schlaepfer-Miller J, Trtikova M. (2012): A controversy re-visited: Is the coccinellid Adalia bipunctata adversely affected by Bt toxins? Environmental Sciences Europe. 24(10).

In 2008/2009, Schmidt and colleagues published a study reporting lethal effects of the microbial Bt toxins Cry1Ab and Cry3Bb on the coccinellid biological control organisms Adalia bipunctata. Based on this study, in concert with over 30 other publications, Mon810 cultivation was banned in Germany in 2009. This triggered two commentaries and one experimental study all published in the journal 'Transgenic Research' that question the scientific basis of the German ban or claim to disprove the adverse effects of the Bt toxins on A. bipunctata reported by Schmidt and colleagues, respectively. This study was undertaken to investigate the underlying reasons for the different outcomes and rebuts the criticism voiced by the two other commentaries. It could be demonstrated that the failure to detect an adverse effect by Alvarez-Alfageme and colleagues is based on the use of a significantly different testing protocol. While Schmidt and colleagues exposed and fed larvae of A. bipunctata continuously, Alvarez-Alfageme and colleagues applied an exposure/recovery protocol. When this exposure/recovery protocol was applied to a highly sensitive target insect, Ostrinia nubilalis, the lethal effect was either significantly reduced or disappeared altogether. When repeating the feeding experiments with the Bt toxin Cry1Ab using a combined protocol of both previous studies, again, a lethal effect on A. bipunctata larvae was observed. ELISA tests with Bt-toxin fed larvae and pupae confirmed ingestion of the toxin. The new data corroborates earlier findings that Cry1Ab toxin increases mortality in A. bipunctata larvae. It was also shown that the different applied testing protocols explained the contrasting results.

5. Bohn T, Traavik T, Primicerio R. (2010): Demographic responses of Daphnia magna fed transgenic Bt-maize. Ecotoxicology. 19(2): 419-430.

The food/feed quality of a variety of genetically modified (GM) maize expressing Cry1Ab Bt-toxin was tested over the life-cycle of Daphnia magna, an arthropod commonly used as model organism in ecotoxicological studies. Demographic responses were compared between animals fed GM or unmodified (UM) near isogenic maize, with and without the addition of predator smell. Age-specific data on survival and birth rates were integrated and analysed using life tables and Leslie matrices. Survival, fecundity and population growth rate (PGR) data generally disfavoured transgenic Bt-maize as feed for D. magna compared to animals fed the unmodified (UM) near isogenic line of maize. Decomposition of age-specific effects revealed that the most important contributions to a reduced PGR in the GM-fed group came from both fecundity and survival differences early in life. We conclude that juvenile and young adult stages are the most sensitive experimental units and should be prioritized in future research. These stages are often omitted in toxicological/ecotoxicological studies and in feeding trials.

6. Kelly D W, Poulin P, Tompkins D M and Townsend C R. (2010): Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. J. Appl. Ecology 47, 498504.

Anthropogenic pollution and disease can cause both lethal and sub-lethal effects in aquatic species but our understanding of how these stressors interact is often not known. Contaminants can reduce host resistance to disease, but whether hosts are impacted at environmentally relevant concentrations is poorly understood. We investigated the independent and combined effects of exposure to the common herbicide glyphosate and the trematode parasite Telogaster opisthorchis on survival and the development of spinal malformations in juvenile Galaxias anomalus, a New Zealand freshwater fish. We then investigated how exposure to a glyphosate concentration gradient (036, 36, 36 mg active ingredient (a.i.) L−1) affected the production and release of the infective cercarial stage of the parasite by its snail intermediate host Potamopyrgus antipodarum. Survival of juvenile fish was unaffected by exposure to glyphosate alone (at an environmentally relevant concentration; 036 mg a.i. L−1) or by T. opisthorchis infection alone. However, simultaneous exposure to infection and glyphosate significantly reduced fish survival. Juvenile fish developed spinal malformations when exposed either to infections alone or to infections and glyphosate, with a trend towards greater severity of spinal malformation after exposure to both stressors. All snails exposed to the highest glyphosate concentration (36 mg a.i. L −1) died within 24 h. Snails exposed to a moderate concentration (36 mg a.i. L−1) produced significantly more T. opisthorchis cercariae than snails in the control group or the low concentration group (036 mg a.i. L−1; the same concentration as in the fish experiment). This is the first study to show that parasites and glyphosate can act synergistically on aquatic vertebrates at environmentally relevant concentrations, and that glyphosate might increase the risk of disease in fish. Our results have important implications when identifying risks to aquatic communities and suggest that threshold levels of glyphosate currently set by regulatory authorities do not adequately protect freshwater systems.

http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2010.01791.x/Abstract

7. Ramirez-Romero R., Desneux N., Decourtye A. Chaffiol A. and Pham-Delègue M.H. (2008): Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? Ecotoxicology and Environ. Safety 70 (3): 327 333.

Genetically modified Bt crops are increasingly used worldwide but side effects and especially sublethal effects on beneficial insects remain poorly studied. Honey bees are beneficial insects for natural and cultivated ecosystems through pollination. The goal of the present study was to assess potential effects of two concentrations of Cry1Ab protein (3 and 5000 ppb) on young adult honey bees. Following a complementary bioassay, our experiments evaluated effects of the Cry1Ab on three major life traits of young adult honey bees: (a) survival of honey bees during sub-chronic exposure to Cry1Ab, (b) feeding behaviour, and (c) learning performance at the time that honey bees become foragers. The latter effect was tested using the proboscis extension reflex (PER) procedure. The same effects were also tested using a chemical pesticide, imidacloprid, as positive reference. The tested concentrations of Cry1Ab protein did not cause lethal effects on honey bees. However, honey bee feeding behaviour was affected when exposed to the highest concentration of Cry1Ab protein, with honey bees taking longer to imbibe the contaminated syrup. Moreover, honey bees exposed to 5000 ppb of Cry1Ab had disturbed learning performances. Honey bees continued to respond to a conditioned odour even in the absence of a food reward. Our results show that transgenic crops expressing Cry1Ab protein at 5000 ppb may affect food consumption or learning processes and thereby may impact honey bee foraging efficiency. The implications of these results are discussed in terms of risks of transgenic Bt crops for honey bees.

http://www.ncbi.nlm.nih.gov/pubmed/18206234

8. Bøhn, T., Primicerio, R., Hessen, D.O. & Traavik, T. (2008): Reduced fitness of Daphnia magna fed a Bt-transgenic maize variety. Archives of Environmental Contamination and Toxicology DOI 10.1007/s00244-008-9150-5

Genetically modified (GM) maize expressing the Bt-toxin Cry1Ab (Bt-maize) was tested for effects on survival, growth, and reproduction of the water flea Daphnia magna, a crustacean arthropod commonly used as a model organism in ecotoxicological studies. In three repeated experiments, D. magna were fed 100% ground maize in suspension, using either GM or isogenic unmodified (UM) maize. D. magna fed GM-maize showed a significantly reduced fitness performance: The mortality was higher, a lower proportion of females reached sexual maturation, and the overall egg production was lower compared to D. magna fed UM isogenic maize. We conclude that the tested variety of Bt-maize and its UM counterpart do not have the same quality as food sources for this widely used model organism. The combination of a reduced fitness performance combined with earlier onset of reproduction of D. magna fed Bt-maize indicates a toxic effect rather than a lower nutritional value of the GM-maize.

http://www.ncbi.nlm.nih.gov/pubmed/18347840

9. Babendreier D, Reichhart B, Romeis J, Bigler F. (2008): Impact of insecticidal proteins expressed in transgenic plants on bumblebee microcolonies. Entomol Exp Appl 126: 148-57

In order to assess the risk that insecticidal transgenic plants may pose for bumblebees, we tested whether Bombus terrestris (L.) (Hymenoptera: Apidae) workers are able to detect insecticidal proteins dissolved in sucrose solution and whether consumption of these proteins will affect survival and offspring production. Feeders containing either Bacillus thuringiensis toxin (Cry1Ab), Kunitz soybean trypsin inhibitor (SBTI), or Galanthus nivalis agglutinin (GNA) were offered to bumblebee colonies at low (0.01% wt/vol for SBTI and GNA, 0.001% for Cry1Ab) and high concentrations (0.1% for SBTI and GNA, 0.01% for Cry1Ab) together with a control (pure sucrose solution) in a glasshouse chamber. No difference was found in the number of visits and the duration of visits among the different concentrations for each of the insecticidal proteins, indicating that bumblebees do not discriminate the compounds. To investigate the impact of the different insecticidal proteins on B. terrestris, microcolonies were established by placing three newly emerged bumblebee workers in wooden boxes. Within a few days, a hierarchy in each microcolony was established and the dominant worker developed its ovaries and laid haploid eggs. Bumblebees were fed with Cry1Ab (0.01%), SBTI, or GNA (both at 0.01 and 0.1%) dissolved in sucrose solution and also fed mixed floral pollen for a maximum period of 80 days. Additionally, microcolonies with three drones each were established to measure individual bee longevity. While the Cry1Ab did not affect microcolony performance, the consumption of SBTI and especially GNA affected survival of B. terrestris workers and drones and caused a significant reduction in the number of offspring. The use of microcolonies appears to be well-suited to measure lethal and sublethal effects of insecticidal proteins expressed in transgenic plants on bumblebees.
University of Minnesota (2008): Extension document 'Bt Corn and European Corn Borer'

http://www.extension.umn.edu/distribution/cropsystems/dc7055.html#ch11

10. Prasifka, P.L., Hellmich, R.L., Prasifka, J.R. & Lewis, L.C. (2007): Effects of Cry1Ab expressing corn anthers on the movement of monarch butterfly larvae. Environmental Entomology 36:228-33

Decreased larval feeding and weight of the monarch butterfly, Danaus plexippus L., have been detected after 4 d of exposure in the laboratory to a high density of Bacillus thuringiensis (Bt)-expressing anthers. One hypothesis is that larvae exposed to Bt anthers exhibit increased wandering, resulting in less feeding and lower weight gain. To test this hypothesis, 2-d-old monarch butterfly larvae exposed to milkweed leaf disks with no anthers, anthers that express Bt (Cry1Ab, event MON810), or other non-Bt anthers were observed using a video-tracking system. As had been shown in previous studies, larvae exposed to Bt anthers fed less and gained less weight than larvae exposed to non-Bt or no anthers, yet there was no evidence of feeding on anthers. Total distance moved, maximum displacement from release point, percentage of time spent moving or near anthers, or mean turn angle did not differ across treatments. However, larvae exposed to Bt anthers spent more time off milkweed leaf disks than those exposed to no anthers and were more likely to move off the leaf than larvae exposed to non-Bt anthers. Results suggest that larvae exposed to Bt anthers behave differently and that ingestion may not be the only way Bt can affect nontarget insects like the monarch butterfly.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Effects+of+Cry1Ab+expressing+corn+anthers+on+the+movement+of+monarch+butterfly+larvae.+Environmental+Entomology+36%3A228-33

11. Marvier M, McCreedy C, Regetz J and Kareiva P (2007): A meta-analysis of effects of Bt cotton and maize on non-target invertebrates. Science. Vol. 316 (5830): 1475-1477.

Although scores of experiments have examined the ecological consequences of transgenic Bacillus thuringiensis (Bt) crops, debates continue regarding the nontarget impacts of this technology. Quantitative reviews of existing studies are crucial for better gauging risks and improving future risk assessments. To encourage evidence-based risk analyses, we constructed a searchable database for nontarget effects of Bt crops. A meta-analysis of 42 field experiments indicates that nontarget invertebrates are generally more abundant in Bt cotton and Bt maize fields than in nontransgenic fields managed with insecticides. However, in comparison with insecticide-free control fields, certain nontarget taxa are less abundant in Bt fields.

Web link: http://www.ncbi.nlm.nih.gov/pubmed/17556584

12. Rosi-Marshall E J, Tank J L, Royer T V, Whiles M R, Evans-White M, Chambers C, Griffiths N A, Pokelsek J and Stephen M L (2007): Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proceedings of the National Academy of Sciences of United States of America, 104(41): 16204-16208.

Corn (Zea mays L.) that has been genetically engineered to produce the Cry1Ab protein (Bt corn) is resistant to lepidopteran pests. Bt corn is widely planted in the midwestern United States, often adjacent to headwater streams. We show that corn byproducts, such as pollen and detritus, enter headwater streams and are subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials showed that consumption of Bt corn byproducts reduced growth and increased mortality of nontarget stream insects. Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystem-scale consequences

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2042185/

13. Douville M, Gagne F, Blaise C. & André C. (2007): Occurrence and persistence of Bacillus thuringiensis (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Eco-toxicology and Environmental Safety 66 (2): 195203.

Genetically modified corn crops and suspensions of Bacillus thuringiensis (Bt) are currently used to control pest infestations of insects of the Lepidoptera family. For this purpose, the cry1Ab gene coding for protein delta-endotoxin derived from B. thuringiensis kurstaki (Btk), which is highly toxic to these insects, was inserted and expressed in corn. The aims of this study were to examine the occurrence and persistence of the cry1Ab gene from Btk and Bt corn in aquatic environments near fields where Bt corn was cultivated. First, an optimal DNA preparation and extraction methodology was developed to allow for quantitative gene analysis by real-time polymerase chain reaction (qPCR) in various environmental matrices. Second, surface water and sediment were spiked in vitro with genomic DNA from Bt or Bt corn to evaluate the persistence of cry1Ab genes. Third, soil, sediment, and water samples were collected before seeding, 2 weeks after pollen release, and after corn harvesting and mechanical root remixing in soils to assess cry1Ab gene content. DNA was extracted with sufficient purity (i.e., low absorbance at 230 nm and absence of PCR-inhibiting substances) from soil, sediment, and surface water. The cry1Ab gene persisted for more than 21 and 40 days in surface water and sediment, respectively. The removal of bacteria by filtration of surface water samples did not significantly increase the half-life of the transgene, but the levels were fivefold more abundant than those in unfiltered water at the end of the exposure period. In sediments, the cry1Ab gene from Bt corn was still detected after 40 days in clay- and sand-rich sediments. Field surveys revealed that the cry1Ab gene from transgenic corn and from naturally occurring Bt was more abundant in the sediment than in the surface water. The cry1Ab transgene was detected as far away as the Richelieu and St. Lawrence rivers (82 km downstream from the corn cultivation plot), suggesting that there were multiple sources of this gene and/or

that it undergoes transport by the water column. Sediment-associated cry1Ab gene from Bt corn tended to decrease with distance from the Bt cornfield. Sediment concentrations of the cry1Ab gene were significantly correlated with those of the cry1Ab gene in surface water (R=0.83;P=0.04). The data indicate that DNA from Bt corn and Bt were persistent in aquatic environments and were detected in rivers draining farming areas.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Occurrence%20and%20persistence%20of%20Bacillus%20thuringiensis%20%28Bt%29%20and%20transgenic%20Bt%20corn%20cry1Ab%20gene%20from%20an%20aquatic%20environment.%20Eco-toxicology%20and%20Environmental%20Safety%2066%20%282%29%3A%20195%E2%80%93203

14. Lang A, Vojtech E. (2006): The effects of pollen consumption of transgenic Bt maize on the common swallowtail, Papilio machaon L. (Lepidoptera, Papilionidae). Basic and Applied Ecology. 2006; 7: 296306

Effects of exposure to maize pollen of event Bt176 (cultivar "Navares") on the larvae of the European common swallowtail (Papilio machaon L.) were studied in the laboratory. First instar larvae were exposed to different pollen densities applied to leaf disks of Pastinaca sativa L. for 48 h. Pollen densities applied in this study were in the range recorded from the field. Larvae which were exposed to higher Bt maize pollen densities consumed more pollen and had a lower survival rate. The LD50 with regard to larvae surviving to adulthood was 13.72 pollen grains consumed by first-instar larva. Uptake of Bt maize pollen led to a reduced plant consumption, to a lower body weight, and to a longer development time of larvae. Effects on pupal weight and duration of the pupal period were present but less pronounced and smaller than effects on larvae. Larvae having consumed Bt-maize pollen as first instars had a lower body weight as adult females and smaller forewings as adult males. We conclude that possible effects of Bt maize on European butterflies and moths must be evaluated more rigorously before Bt maize should be cultivated over large areas.

15. Obrist L.B., Dutton A., Romeis J. & Bigler F. (2006): Biological activity of Cry1Abtoxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator Chrysoperla carnea. BioControl 51: 31-48.

A major concern regarding the deployment of insect resistant transgenic plants is their potential impact on non-target organisms, in particular on beneficial arthropods such as predators. To assess the risks that transgenic plants pose to predators, various experimental testing systems can be used. When using tritrophic studies, it is important to verify the actual exposure of the predator, i.e., the presence of biologically active toxin in the herbivorous arthropod (prey). We therefore investigated the uptake of Cry1Ab toxin by larvae of the green lacewing (Chrysoperla carnea (Stephens); Neuroptera: Chrysopidae) after consuming two Bt maize-fed herbivores (Tetranychus urticae Koch; Acarina: Tetranychidae and Spodoptera littoralis (Boisduval); Lepidoptera: Noctuidae) by means of an immunological test (ELISA) and the activity of the Cry1Ab toxin following ingestion by the herbivores. Moreover, we compared the activity of Cry1Ab toxin produced by Bt maize to that of purified toxin obtained from transformed Escherichia coli, which is recommended to be used in toxicity studies. The activity of the toxin was assessed by performing feeding bioassays with larvae of the European corn borer (Ostrinia nubilalis (Hübner); Lepidoptera: Crambidae), the target pest of Cry1Ab expressing maize. ELISA confirmed the ingestion of Bt toxin by C. carnea larvae when fed with either of the two prey species and feeding bioassays using the target pest showed that the biological activity of the Cry1Ab toxin is maintained after ingestion by both herbivore species. These findings are discussed in the context of previous risk assessment studies with C. carnea. The purified Cry1Ab protein was more toxic to O. nubilalis compared to the plant-derived Cry1Ab toxin when applied at equal concentrations according to ELISA measurements. Possible reasons for these findings are discussed.

http://link.springer.com/article/10.1007%2Fs10526-005-2936-8

16. Hilbeck A. & Schmidt J.E.U. (2006): Another view on Bt proteins how specific are they and what else might they do? Biopesticides International. 2: 1-50.

The entomopathogenic bacterium Bacillus thuringiensis (Bt) and its toxins are extensively used for pest control purposes in agriculture, forestry and public health programmes since the 1930. In addition to spray formulations, transgenic plants containing Bt genes for the expression of the toxins (Bt plants) are commercially available since the mid 1990s and are grown on an increasing percentage of the global agricultural area. A main reason for the importance of Bt as a pesticide is the assumed environmental safety concluded from the high specificity of its endotoxins (Cry proteins) towards a limited number of target organisms, mostly distinct groups of pest insects. While the mode of action of the Cry toxins in these susceptible target insects is well studied, Bt experts claim that several details are still not understood well enough. Although there is considerable experience with the application and the environmental safety of Bt sprays, a number of research papers were published in the past that did report adverse effects on non-target organisms. These and the widespread use of transgenic Bt plants stimulated us to review the published laboratory feeding studies on effects of Bt toxins and transgenic Bt plants on non-target invertebrates. We describe those reports that documented adverse effects in non-target organisms in more detail and focus on one prominent example, the green lacewing, Chrysoperla carnea. Discussing our findings in the context of current molecular studies, we argue firstly that the evidence for adverse effects in non-target organisms is compelling enough that it would merit more research. We further conclude from our in-depth analysis that the published reports studying the effects of Bt toxins from Bt pesticides and transgenic Bt plants on green lacewing larvae provide complementary and not contradictory data. And, finally, we find that the key experiments explaining the mode of action not only in this particular affected non-target species but also in most other affected non-target species are still missing. Considering the steadily increasing global production area of Bt crops, it seems prudent to thoroughly understand how Bt toxins might affect non-target organisms.

17. Dolezel, M., Heissenberger, A. & Gaugitsch, H. (2006): Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance. Bundesministerium für Gesundheit und Frauen, Sektion IV Radetzkystraße 2, 1031 Wien.

This review evaluates scientific studies published in peer-review journals during the last 3-4 years that considered ecological effects of insect resistant (Bt) and herbicide tolerant maize. The majority of the scientific studies deal with ecological effects of maize containing the Cry1Ab toxin. In contrast, very few studies are available that considered other Cry toxins used in Bt maize such as Cry3Bb1 or Cry1F. Studies dealing with the Cry1Ab toxin relate to effects on non-target Lepidoptera which were the first non-target effects confirmed for Bt176 maize. New studies confirm these adverse effects of Bt176 pollen to non-target Lepidoptera but show that also MON810 and Bt11 maize pollen or anthers may adversely affect lepidopteran larvae especially under prolonged exposure. Additive effects can be expected when larvae are exposed to a combination of Bt pollen and anthers containing the Cry1Ab toxin. Generally, effects on lepidopteran larvae are shown to be species and age-specific. Exposure of non-target lepidopteran larvae to Bt maize pollen under field conditions can be highly variable and is still unknown for the majority of European butterfly species. Published studies on ecological effects of Bt maize containing the Cry1Ab toxin also deal with impacts on other non-target organisms than Lepidoptera, such as herbivorous and predatory arthropods. A large range of herbivorous or predatory species have been shown to contain the Cry1Ab toxin when exposed to Bt maize in the field and adverse impacts on some species were confirmed mostly in laboratory studies. There is a definite need for standardization of laboratory feeding assays or tritrophic experiments with non-target herbivores and predators in order to enable the comparability of these studies. It is unclear if these adverse effects which were observed in the laboratory can be also translated to field conditions. Results on nontarget arthropod abundance in Bt and non-Bt maize fields are inconsistent and adverse effects are mostly restricted to

single years or locations or certain species. Methodological flaws in the experimental design and few replications make it unlikely to detect small abundance effects of these non-target organisms in most field studies. According to the studies currently available major effects on non-target species abundance due to Bt maize cultivation seem to be rather unlikely. Therefore the emphasis of further studies should be on the detection of subtle and long-term effects to non-target organisms. Adverse effects of Bt maize containing the Cry1Ab toxin on parasitoids and hyperparasitoids have been shown and are most likely due to indirect and host-mediated effects. Reports on soil persistence and insecticidal activity of Cry1Ab toxins are still controversial although differences in the experimental design or methods used explain to some extent the different results obtained. Some adverse effects of Bt maize on different soil organisms are indicated but confirmation of these indications is still needed. Nevertheless decomposition is most likely different between Bt and non-Bt maize containing the Cry1Ab toxin and was confirmed even for different Bt plant species which is probably the result of differences in lignification patterns between Bt and non-Bt maize. In ontrast to non-target studies of Bt maize containing the Cry1Ab toxin only few studies have so far evaluated in depth non-target effects of the Cry3Bb1 containing maize. These studies give only few indications for consistent or major effects of this toxin on non-target organisms. Neither laboratory studies nor field experiments considering effects of the Cry1F toxin or the insecticidal toxins Cry34Ab1 and Cry35Ab1 on non-target herbivores, predators or soil organisms are so far available. Currently no evidence is available confirming negative impacts of Cry1Ab, Cry3Bb1 and Cry1F toxins on pollinators such as honey bees. Only few studies are currently available that consider ecological effects of herbicide tolerant maize. The results of the British Farm Scale Evaluations were re-analysed recently. Exclusion of pre-emergence atrazine treatments from the analyses resulted in fewer positive effects of the herbicide tolerant maize fields compared to the non transgenic maize fields than reported previously both for weeds and arthropods. Other studies have, however, shown that the continuous use of glyphosate can change weed communities. Other studies considering herbicide tolerant maize predict the increase of

infections by root pathogens due to the delay of the herbicide application. The occurrence of herbicide tolerant weeds has been observed since the large-scale introduction of herbicide tolerant crops and a further increase in abundance and frequency of herbicide resistant weeds is expected to occur.

http://bmg.gv.at/cms/home/attachments/5/6/2/CH1052/CMS113445751 5326/cms1200662494442_literaturstudie_mais_endbericht.pdf

18. Snow AA, Andow D A, Gepts P, Hallerman E M, Power A, Tiedje J M and Wolfenbarger LL (2005): Genetically engineered organisms and the environment: Current status and recommendations. Ecological Applications 15 (2): 377-404.

The Ecological Society of America has evaluated the ecological effects of current and potential uses of field-released genetically engineered organisms (GEOs), as described in this Position Paper. Some GEOs could play a positive role in sustainable agriculture, forestry, aquaculture, bioremediation, and environmental management, both in developed and developing countries. However, deliberate or inadvertent releases of GEOs into the environment could have negative ecological effects under certain circumstances. Possible risks of GEOs could include: (1) creating new or more vigorous pests and pathogens; (2) exacerbating the effects of existing pests through hybridization with related transgenic organisms; (3) harm to nontarget species, such as soil organisms, non-pest insects, birds, and other animals; (4) disruption of biotic communities, including agro-ecosystems; and (5) irreparable loss or changes in species diversity or genetic diversity within species. Many potential applications of genetic engineering extend beyond traditional breeding, encompassing viruses, bacteria, algae, fungi, grasses, trees, insects, fish, and shellfish. GEOs that present novel traits will need special scrutiny with regard to their environmental effects. The Ecological Society of America supports the following recommendations. (1) GEOs should be designed to reduce environmental risks. (2) More extensive studies of the environmental benefits and risks associated with GEOs are needed. (3) These effects should be evaluated relative to appropriate baseline scenarios. (4) Environmental release of GEOs should be prevented if scientific knowledge about possible risks is clearly inadequate. (5) In some cases, post-release monitoring will be needed to identify, manage, and mitigate environmental risks. (6) Science-based regulation should subject all transgenic organisms to a similar risk assessment framework and should incorporate a cautious approach, recognizing that many environmental effects are GEO- and site-specific.

(7) Ecologists, agricultural scientists, molecular biologists, and others need broader training and wider collaboration to address these recommendations. In summary, GEOs should be evaluated and used within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management. The Ecological Society of America is committed to providing scientific expertise for evaluating and predicting the ecological effects of field-released transgenic organisms.

http://www.jstor.org/discover/10.2307/4543362?uid=3738256&uid=2129&uid=2&uid=70&uid=4&sid=21101649652753

19. Clark B.W, Phillips T.A. & Coats J.R. (2005): Environmental fate and effects of Bacillus thuringiensis (Bt) proteins from transgenic crops: a review. Journal of Agricultural and Food Chemistry 53: 4643-4653.

This paper reviews the scientific literature addressing the environmental fate and nontarget effects of the Cry protein toxins from Bacillus thuringiensis (Bt), specifically resulting from their expression in transgenic crops. Published literature on analytical methodologies for the detection and quantification of the Cry proteins in environmental matrices is also reviewed, with discussion of the adequacy of the techniques for determining the persistence and mobility of the Bt proteins. In general, assessment of the nontarget effects of Bt protein toxins indicates that there is a low level of hazard to most groups of nontarget organisms, although some investigations are of limited ecological relevance. Some published reports on the persistence of the proteins in soil show short half-lives, whereas others show low-level residues lasting for many months. Improvements in analytical methods will allow a more complete understanding of the fate and significance of Bt proteins in the environment.

http://pubs.acs.org/doi/abs/10.1021/jf040442k

20. Harwood J.D, Wallin W.G. & Obrycki J.J. (2005): Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Molecular Ecology 14: 2815-2823.

The planting of transgenic crops expressing Bacillus thuringiensis endotoxins is widespread throughout the world; the prolific increase in their application exposes nontarget organisms to toxins designed to control pests. To date, studies have focused upon the effects of Bt endotoxins on specific herbivores and detritivores, without consideration of their persistence within arthropod food webs. Here, we report the first quantitative field evaluation of levels of Bt endotoxin within nontarget herbivores and the uptake by higher order arthropods. Antibody-based assays indicated significant quantities of detectable Cry1Ab endotoxin within nontarget herbivores which feed on transgenic corn (including the corn flea beetle, Chaetocnema pulicaria, Japanese beetle, Popillia japonica and southern corn rootworm, Diabrotica undecimpunctata howardi). Furthermore, arthropod predators (Coccinellidae, Araneae, and Nabidae) collected from these agroecosystems also contained significant quantities of Cry1Ab endotoxin indicating its movement into higher trophic levels. This uptake by predators is likely to have occurred by direct feeding on plant material (in predators which are facultatively phytophagous) or the consumption of arthropod prey which contained these proteins. These data indicate that long-term exposure to insecticidal toxins occurs in the field. These levels of exposure should therefore be considered during future risk assessments of transgenic crops to nontarget herbivores and arthropod predators.

http://www.uky.edu/~jdharw2/harwoodetal2005b.pdf

21. Poerschmann J, Gathmann A, Augustin J, Langer U and Górecki T. (2005): Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize Characterization of lignin patterns. Journal of Environmental Quality 34: 1508-1518.

Transformation of crops, including maize (Zea mays L.), with the cry1Ab gene from Bacillus thuringiensis to combat lepidopteran pests results in pleiotropic effects regarding lignin biosynthesis. Lignin patterns in stems and leaves of two genetically modified Bt-maize varieties (Novelis T and Valmont T) were studied along with their non-Bt near-isolines (Nobilis and Prelude, respectively). Molecular-level based thermochemolysis using tetramethylammonium hydroxide (TMAH) in combination with gas chromatography-mass spectrometry (GC-MS) was used to quantitate the total lignin contents and to identify monomeric lignin subunits including p-hydroxyphenyl (P), guaiacyl (G), and syringyl (S) moieties. The results were supplemented and confirmed by cupric oxide oxidation. The stems of the transgenic lines had higher concentrations of total lignin than the respective isogenic lines: Valmont T/Prelude by 18% and Novelis T/Nobilis by 28%. In contrast, differences in the total lignin concentration of leaves between the transgenic and the respective near-isogenic lines were marginal. There were significant modifications in the ratio of p-hydroxyphenyl/guaiacyl/syringyl molecular marker units of stem lignin between transgenic and isogenic lines. The guaiacyl units (in particular the G18 marker) accounted chiefly for the higher total lignin contents in the transgenic lines. The leaf lignin patterns did not show significant differences in molecular markers between isogenic and transgenic lines. TMAH-induced thermochemolysis--conducted in both the on-line and off-line modes--provided detailed information on the molecular composition of lignin, thus proving superior to the established "wet chemistry" methods of lignin determination.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Molecular+composition+of+leaves+and+stems+of+genetically+modified+Bt+and+near-isogenic+non-Bt+maize+%E2%80%93+Characterization+of+lignin+ patterns.+Journal+of+Environmental+Quality+34%3A+1508-1518

22. Relyea R.A., Schoeppner N. M., Hoverman J.T. (2005): Pesticides and amphibians: the importance of community context. Ecological Applications 15: 11251134.

The widespread application of pesticides has attracted the attention of ecologists as we struggle to understand the impacts of these chemicals on natural communities. While we have a large number of laboratory-based, single-species studies of pesticides, such studies can only examine direct effects. However, in natural communities, species can experience both direct and indirect effects. We conducted an outdoor mesocosm experiment on aquatic communities containing three tadpole species (Hyla versicolor, Bufo americanus, and Rana pipiens), zooplankton, and algae. We then manipulated a factorial combination of predators (no predators; newts, Notophthalmus viridescens; and larval beetles, Dytiscus sp.) and pesticides (no pesticides, the insecticide malathion, and the herbicide Roundup). We found that Roundup (1.3 mg of active ingredient/L) had substantial direct negative effects on the tadpoles, reducing total tadpole survival and biomass by 40%. However, Roundup had no indirect effects on the amphibian community via predator survival or algal abundance. Malathion (0.3 mg/L) had few direct effects on the tadpoles. Malathion caused no indirect effects with one of the predators (red-spotted newts) but caused substantial positive effects on amphibians (a five-fold increase in total tadpole survival and biomass) due to the sensitivity of the predatory beetles to the insecticide. Thus, while high concentrations of malathion can directly kill larval anurans, more ecologically relevant concentrations can have large positive effects in mesocosms by removing predatory insects. These results make it clear that pesticides can have both direct and indirect effects in natural communities and that these effects critically depend upon the composition of the community.

http://online.sfsu.edu/carlosd/contaminant/relyea%20commun%20context%20ecolapps%2005.pdf

23. Relyea, R.A. (2005): The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecological Applications, 15 (4): 11181124.

The global decline in amphibian diversity has become an international environmental problem with a multitude of possible causes. There is evidence that pesticides may play a role, yet few pesticides have been tested on amphibians. For example, Roundup is a globally common herbicide that is conventionally thought to be nonlethal to amphibians. However, Roundup has been tested on few amphibian species, with existing tests conducted mostly under laboratory conditions and on larval amphibians. Recent laboratory studies have indicated that Roundup may be highly lethal to North American tadpoles, but we need to determine whether this effect occurs under more natural conditions and in post-metamorphic amphibians. I assembled communities of three species of North American tadpoles in outdoor pond mesocosms that contained different types of soil (which can absorb the pesticide) and applied Roundup as a direct overspray. After three weeks, Roundup killed 96100% of larval amphibians (regardless of soil presence). I then exposed three species of juvenile (post-metamorphic) anurans to a direct overspray of Roundup in laboratory containers. After one day, Roundup killed 6886% of juvenile amphibians. These results suggest that Roundup, a compound designed to kill plants, can cause extremely high rates of mortality to amphibians that could lead to population declines.

24. Relyea, R.A. (2005): The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Appl. 15, 618627.

Pesticides constitute a major anthropogenic addition to natural communities.Inaquatic communities, a great majority of pesticide impacts are determined from singlespecies experiments conducted under laboratory conditions. Although this is an essential protocol to rapidly identify the direct impacts of pesticides on organisms, it prevents an assessment of direct and indirect pesticide effects on organisms embedded in their natural ecological contexts. In this study, I examined the impact of four globally common pesticides (two insecticides, carbaryl [Sevin] and malathion; two herbicides, glyphosate [Roundup] and 2,4-D) on the biodiversity of aquatic communities containing algae and 25 species of animals. Species richness was reduced by 15% with Sevin, 30% with malathion, and 22% with Roundup, whereas 2,4-D had no effect. Both insecticides reduced zooplankton diversity by eliminating cladocerans but not copepods (the latter increased in abundance). The insecticides also reduced the diversity and biomass of predatory insects and had an apparent indirect positive effect on several species of tadpoles, but had no effect on snails. The two herbicides had no effects on zooplankton, insect predators, or snails. Moreover, the herbicide 2,4-D had no effect on tadpoles. However, Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when nontarget organisms are examined under ecologically relevant conditions.

http://people.sc.fsu.edu/~pbeerli/BSC3052/restricted/papers/relyea-2005.pdf

25. Dively G.P., Rose R., Sears M.K., Hellmich R.L., Stanley-Horn D.E., Calvin D.D. Russo J.M. & P.L. Anderson. (2004): Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab expressing corn during anthesis. Environmental Entomology 33: 1116-1125.

Effects on monarch butterfly, Danaus plexippus L., after continuous exposure of larvae to natural deposits of Bacillus thuringiensis (Bt) and non-Bt pollen on milkweed, were measured in five studies. First instars were exposed at 3-4 and 6-7 d after initial anthesis, either directly on milkweed plants in commercial cornfields or in the laboratory on leaves collected from milkweeds in corn plots. Pollen exposure levels ranging from 122 to 188 grains/cm2/d were similar to within-field levels that monarch butterfly populations might experience in the general population of cornfields. Results indicate that 23.7% fewer larvae exposed to these levels of Bt pollen during anthesis reached the adult stage. A risk assessment procedure used previously was updated with a simulation model estimating the proportion of second-generation monarch butterflies affected. When considered over the entire range of the Corn Belt, which represents only 50% of the breeding population, the risk to monarch butterfly larvae associated with long-term exposure to Bt corn pollen is 0.6% additional mortality. Exposure also prolonged the developmental time of larvae by 1.8 d and reduced the weights of both pupae and adults by 5.5%. The sex ratio and wing length of adults were unaffected. The ecological significance of these sublethal effects is discussed relative to generation mortality and adult performance.

http://www.researchgate.net/publication/233611533_Effects_on_Monarch_Butterfly_Larvae_%28Lepidoptera_Danaidae%29_After_Continuous_Exposure_to_Cry1Ab-Expressing_Corn_During_Anthesis

26. Brodsgaard H F, Brodsgaard C J, Hansen H & Lovei G L (2003): Environmental risk assessment of transgene products using honey bee (Apis mellifera) larvae. Apidologie 34: 139145.

An environmental concern regarding the cultivation of transgenic crop plants is their effect on non-target organisms. Honey bees are obvious non-target arthropods to be included in a risk assessment procedure but due to their complex social behaviour, testing transgene products on individual bees is not possible in bee colonies. We employed a laboratory larval rearing technique to test the impacts of such transgene products on honey bees. A serine proteinase inhibitor (Kunitz Soybean Trypsin Inhibitor, SBTI), that is a source of insect resistance in transgenic plants, was used as a model insecticidal protein on honey bee larvae reared individually in the laboratory. The addition of 1.0% SBTI (w:w of total protein) to the larval diet created significant additional larval mortality, slowed juvenile development and significantly decreased adult body mass. Our results suggest that the larval rearing technique can be used to monitor direct side-effects of transgene products on individual honey bee larvae.

http://dx.doi.org/10.1051/apido:2003003

27. Dutton A, Klein H, Romeis J, Bigler F. (2002): Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator Chrysoperia carnea. Ecol Entomol 27: 441-7.

Chrysoperla carnea is an important predatory insect in maize. To assess the ecological effects of Bt-maize, expressing the Cry1Ab protein, on larvae of this predator, the following factors were examined: (1) the performance of three prey herbivores (Rhopalosiphum padi, Tetranychus urticae, and Spodoptera littoralis) on transgenic Bt and non-transgenic maize plants; (2) the intake of the Cry1Ab toxin by the three herbivores; and (3) the effects on C. carnea when fed each of the prey species. The intrinsic rate of natural increase (rm) was used as a measure of performance for R. padi and T. urticae. No difference in this parameter was observed between herbivores reared on Bt or non-transgenic plants. In contrast, a higher mortality rate and a delay in development were observed in S. littoralis larvae when fed Bt-maize compared with those fed the control maize plants. The ingestion of Cry1Ab toxin by the different herbivores was measured using an immunological assay (ELISA). Highest amounts of Cry1Ab toxin were detected in T. urticae, followed by S. littoralis, and only trace amounts detected in R. padi. Feeding C. carnea with T. urticae, which were shown to contain the Cry1Ab toxin, or with R. padi, which do not ingest the toxin, did not affect survival, development, or weight of C. carnea. In contrast, a significant increase in mortality and a delay in development were observed when predators were fed S. littoralis larvae reared on Bt-maize. A combined interaction of poor prey quality and Cry1Ab toxin may account for the negative effects observed on C. carnea when fed S. littoralis. The relevance of these findings to the ecological risks of Bt-maize on C. carnea is discussed.

Wang Z-H, Shu Q-Y, Cui H-R, Xu M-K, Xie X-B & Y-W Xia (2002): The effect of Bt transgenic rice flour on the development of silkworm larvae and the sub-microstructure of its midgut. Scientia Agricultura Sinica 35: 714-718. NO ABSTRACT AVAILABLE.

28. Saxena D and Stotzky G. (2001): Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88: 1704-1706.

Bt corn has been genetically modified to express the Cry1Ab protein of Bacillus thuringiensis to kill lepidopteran pests. Fluorescence microscopy and staining with toluidine blue indicated a higher content of lignin in the vascular bundle sheaths and in the sclerenchyma cells surrounding the vascular bundle in all ten Bt corn hybrids, representing three different transformation events, studied than of their respective non-Bt isolines. Chemical analysis confirmed that the lignin content of all hybrids of Bt corn, whether grown in a plant growth room or in the field, was significantly higher (3397% higher) than that of their respective non-Bt isolines. As lignin is a major structural component of plant cells, modifications in lignin content may have ecological implications.

http://www.amjbot.org/content/88/9/1704.short

29. Stanley-Horn D.E, Dively GP, Hellmich RL, Mattila HR, Sears MK, Rose R, Jesse LC, Losey JE, Obrycki JJ and Lewis L (2001): Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proceedings of the National Academy of Sciences 98 (21): 11931-11936.

Survival and growth of monarch larvae, Danaus plexippus (L.), after exposure to either Cry1Ab-expressing pollen from three Bacillus thuringiensis (Bt) corn (Zea mays L.) events differing in toxin expression or to the insecticide, lambda-cyhalothrin, were examined in field studies. First instars exposed to low doses (approximately 22 grains per cm(2)) of event-176 pollen gained 18% less weight than those exposed to Bt11 or Mon810 pollen after a 5-day exposure period. Larvae exposed to 67 pollen grains per cm(2) on milkweed leaves from within an event-176 field exhibited 60% lower survivorship and 42% less weight gain compared with those exposed to leaves from outside the field. In contrast, Bt11 pollen had no effect on growth to adulthood or survival of first or third instars exposed for 5 days to approximately 55 and 97 pollen grains per cm(2), respectively. Similarly, no differences in larval survivorship were observed after a 4-day exposure period to leaves with 504-586 (within fields) or 18-22 (outside the field) pollen grains per cm(2) collected from Bt11 and non-Bt sweet-corn fields. However, survivorship and weight gain were drastically reduced in non-Bt fields treated with lambda-cyhalothrin. The effects of Bt11 and Mon810 pollen on the survivorship of larvae feeding 14 to 22 days on milkweeds in fields were negligible. Further studies should examine the lifetime and reproductive impact of Bt11 and Mon810 pollen on monarchs after long-term exposure to naturally deposited pollen.

http://www.ncbi.nlm.nih.gov/pubmed/11559839

30. Zangerl A R, McKenna D, Wraight C L, Carroll M, Ficarello P, Warner R and M R Berenbaum (2001): Effects of exposure to event 176 Bacillus thuringiensis corn pollen on monarch and black swallowtail caterpillars under field conditions. Proc. National Academy of Science USA 98: 11908-11912.

The widespread planting of corn genetically modified to produce Bacillus thuringiensis endotoxin has led to speculation that pollen from these fields might adversely affect nearby nontarget lepidopterans. A previous study of Bt corn engineered with Monsanto event 810 failed to detect an effect of pollen exposure on the black swallowtail, Papilio polyxenes, in either the field or the laboratory. Here, we report results of a field study investigating the impact of exposure to pollen from a Bt corn hybrid containing Novartis event 176 on two species of Lepidoptera, black swallowtails and monarch butterflies, Danaus plexippus. Nearly half of the 600 monarch larvae died within the first 24 h; this and subsequent mortality was not associated with proximity to Bt corn and may have been due in part to predation. Survivorship of black swallowtails was much higher than that of the monarchs and was also independent of proximity to the transgenic corn. However, despite five rainfall events that removed much of the pollen from the leaves of their host plants during the experiment, we observed a significant reduction in growth rates of black swallowtail larvae that was likely caused by pollen exposure. These results suggest that Bt corn incorporating event 176 can have adverse sublethal effects on black swallowtails in the field and underscore the importance of event selection in reducing environmental impacts of transgenic plants.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Effects+of+exposure+to+event+176+Bacillus+thuringiensis+corn+pollen+on+monarch+and+black+swallowtail+caterpillars+under+field+conditions

31. Hansen Jesse L and Obrycki JJ (2000): Field deposition of Bt transgenic corn pollen lethal effects on the monarch butterfly. J. Oecologia. Vol. 125 (2): 241-8.

We present the first evidence that transgenic Bacillus thuringiensis (Bt) corn pollen naturally deposited on Asclepias syriaca; common milkweed, in a corn field causes significant mortality of Danaus plexippus L. (Lepidoptera: Danaidae) larvae. Larvae feeding for 48 h on A. syriaca plants naturally dusted with pollen from Bt corn plants suffered significantly higher rates of mortality at 48 h (20±3%) compared to larvae feeding on leaves with no pollen (3±3%), or feeding on leaves with non-Bt pollen (0%). Mortality at 120 h of D. Plexippus larvae exposed to 135 pollen grains/cm2 of transgenic pollen for 48 h ranged from 37 to 70%. We found no sub-lethal effects on D. plexippus adults reared from larvae that survived a 48-h exposure to three concentrations of Bt pollen. Based on our quantification of the wind dispersal of this pollen beyond the edges of agricultural fields, we predict that the effects of transgenic pollen on D. plexippus may be observed at least 10 m from transgenic field borders. However, the highest larval mortality will likely occur on A. syriaca plants in corn fields or within 3 m of the edge of a transgenic corn field. We conclude that the ecological effects of transgenic insecticidal crops need to be evaluated more fully before they are planted over extensive areas.

http://adamoliverbrown.com/wp-content/uploads/2010/01/5-BT-corn-and-monarch.pdf

32. Newmaster S G, Bell F W and Vitt D H. (1999): The effects of glyphosate and triclopyr on common bryophytes and lichens in northwestern Ontario. Can. Jour. Forest Research 29, 11011111.

The effects of two silvicultural herbicides (VisionR, ReleaseR) on bryophytes and lichens were studied in a harvested boreal mixedwood ecosystem. A completely randomized design with 115 plots of 1 m2 allowed direct comparison between herbicides and their effects on community dynamics. Regression models were used to analyze the relationship between herbicide application rates (0.71-6.72 kg active ingredient/ha) and changes in bryophyte and lichenabundance and species richness for 2 years following herbicide application. Results showed that bryophyte and lichen abundance and species richness decreased after herbicide treatments. In general, herbicide applications reduced the diversity of forest mesophytes and weedy colonizers to an ecosystem with only a few species of colonizers. A combination of clustering techniques and ANOVA were used to divide bryophytes and lichens into three ecologically defined response groups: herbicide-tolerant colonizers, semi tolerant long-term stayers from dry open forest, and sensitive forest mesophytes.

http://connection.ebscohost.com/c/articles/8053050/effects-glyphosate-triclopyr-common-bryophytes-lichens-northwestern-ontario

33. Losey J.E., Rayor LS and Carter ME (1999): Transgenic pollen harms monarch larvae. Nature (May 20 1999) 399 (6733): 214.

Although plants transformed with genetic material from the bacterium Bacillus thuringiensis (Bt) are generally thought to have negligible impact on non-target organisms, Bt corn plants might represent a risk because most hybrids express the Bt toxin in pollen, and corn pollen is dispersed over at least 60 metres by wind. Corn pollen is deposited on other plants near corn fields and can be ingested by the non-target organisms that consume these plants. In a laboratory assay we found that larvae of the monarch butterfly, Danaus plexippus, reared on milkweed leaves dusted with pollen from Bt corn, ate less, grew more slowly and suffered higher mortality than larvae reared on leaves dusted with untransformed corn pollen or on leaves without pollen.

http://www.nature.com/nature/journal/v399/n6733/abs/399214a0.html

34. Hilbeck A., Baumgartner M, Fried PM and Bigler F (1998): Effects of transgenic Bacillus thuringiensis corn-fed prey on mortality and development time of immature Chrysoperla carnea. Environmental Entomology 276: 480-487.

Laboratory feeding experiments were conducted to determine the effects of Bt-fed herbivores (Ostrinia nubilalis and Spodoptera littoralis) on the predator Chrysoperla carnea. Bt corn (HD-1) coding for CryIAb and an isogenic strain were grown in greenhouses and used when they reached 50-80 cm (5-8 leaf stage). Mortality of chrysopid larvae raised on Bt-fed prey was significantly higher (67%) compared to those raised on Bt-free prey (37%). No differences were observed between chrysopid larvae raised on Bt-fed O. nubilalis or S. littoralis. Chrysopid larvae developmental time was prolonged when raised on Bt-fed O. nubilalis but not Bt-fed S. littoralis.

http://www.ent.iastate.edu/toxicology/node/221

35. Tate T M, Spurlock J O and Christian F A. (1997): Effect of glyphosate on the development of Pseudosuccinea columella snails. Arch. Environ. Contam. Toxicol. 33, 286289.

Glyphosate (Roundup) is one of the most commonly used broad-spectrum herbicides with little to no hazard to animals, man, or the environment. Due to its widespread use, there is continuous contamination of the environment in both soil and water with this herbicide. There is a paucity of long-term exposure studies with sublethal concentrations of glyphosate on aquatic snails. This study was developed to determine the effects of sublethal concentrations of glyphosate on development and survival of Pseudosuccinea columella (intermediate snail host of Fasciola hepatica). This was assessed by continuously exposing three successive generations of snails to varying concentrations (0.110 mg/L) of glyphosate. Glyphosate had little effect on the first- and second-generation snails. However, third-generation snail embryos exposed to 1.0 mg/L glyphosate developed much faster than other embryos exposed to 0.1 mg/L, 10 mg/L, and 0 mg/L (control). Hatching was inhibited at 10 mg/L and inhibited slightly at 0.1 mg/L. The egg-laying capacity was increased in snails exposed to 0.1 and 10 mg/L. Abnormalities and polyembryony were observed in snails exposed to 0.1 and 10 mg/L. These results indicate that glyphosate does affect snail reproduction and development. This, in turn, could possibly have an effect on the population dynamics of F. hepatica, which could result in increased infections in animals, including man.

http://link.springer.com/article/10.1007%2Fs002449900255?LI=true

36. Springett J A and Gray R A J. (1992): Effect of repeated low doses of biocides on the earthworm Aporrectodea caliginosa in laboratory culture. Soil Biol. Biochem. 24, 17391744.

The growth rates of Aporrectodea caliginosa (Savigny) were measured over a 100-day period in soil in culture chambers which were treated with common biocides singly and in combination. The biocides used were: the fungicide Captan, the herbicide, Glyphosate and the insecticide, Azinphos-methyl. The biocides were applied at intervals of 14 days and each treatment was replicated six times. The results arc variable, all biocides depressed growth when applied alone but some combinations reduced the effect of other biocides. Azinphos-methyl and Glyphosate applied alone, reduced growth the most over the 100 days and at all rates of application. Azinphos-methyl applied at the highest rate killed worms. Captan applied alone had the least effect on growth and mortality. In combination, Glyphosate and Captan had a lesser effect than Glyphosate alone. Azinphos-methyl and Captan had less effect than Azinphos-methyl alone. After 100 days the combination of all three biocides reduced growth to the same degree as Glyphosate alone.

http://europepmc.org/Abstract/AGR/IND93024766

37. Santillo D J, Brown P W and Leslie D M. (1989): Response of songbirds to glyphosate induced habitat changes on clearcuts. J. Wildlife Management 53, 6471.

We examined breeding bird populations and habitats on glyphosate (nitrogen-phosphonomethyl glycine) (Roundup, Monsanto, St. Louis, Mo.)-treated and untreated clearcuts in north-central Maine. Treatment of clearcuts with glyphosate herbicide reduced the complexity of vegetation through 3 years post-treatment compared to untreated clearcuts. Total numbers of birds, common yellowthroats (Geothlypis trichas), Lincoln's sparrows (Melospiza lincolnii), and alder flycatchers (Empidonax alnorum) were less abundant ($P < 0.05$) on treated clearcuts than on untreated clearcuts. Songbird densities were correlated with habitat complexity, especially hardwood regeneration, foliage height diversity (FHD), and vegetation height. Leaving untreated patches of vegetation and staggering herbicide treatments on large clearcuts will maintain bird populations similar to those on untreated clearcuts.

http://www.jstor.org/discover/10.2307/3801307?uid=3738256&uid=2&uid=4&sid=21101649971863

SUPER WEEDS

1. Sarah M. Ward, Theodore M. Webster, and Larry E. Stecke (2013): Palmer Amaranth (Amaranthus palmeri): A Review. Weed Technology 27(1) :12-27

In little over 20 yrs, Palmer amaranth has risen from relative obscurity to its current status as one of the most widespread, troublesome, and economically damaging agronomic weeds in the southeastern U.S. Numerous factors have enabled Palmer amaranth to become such a dominant and difficult-to-control weed, including its rapid growth rate, high fecundity, genetic diversity, ability to tolerate adverse conditions, and its facility for evolving herbicide resistance. It is both a serious threat to several U.S. cropping systems and a fascinating model weed. In this paper, we review the growing body of literature on Palmer amaranth to summarize the current state of knowledge on the biology, agricultural impacts, and management of this weed, and we suggest future directions for research.

2. Mortensen DA, Egan JF, Maxwell BD, Ryan MR, Smith RG. (2012): Navigating a critical juncture for sustainable weed management. BioScience. 62(1): 75-84

Agricultural weed management has become entrenched in a single tacticherbicideresistant cropsand needs greater emphasis on integrated practices that are sustainable over the long term. In response to the outbreak of glyphosate-resistant weeds, the seed and agrichemical industries are developing crops that are genetically modified to have combined resistance to glyphosate and synthetic auxin herbicides. This technology will allow these herbicides to be used over vastly expanded areas and will likely create three interrelated challenges for sustainable weed management. First, crops with stacked herbicide resistance are likely to increase the severity of resistant weeds. Second, these crops will facilitate a significant increase in herbicide use, with potential negative consequences for environmental quality. Finally, the short-term fix provided by the new traits will encourage continued neglect of public research and extension in integrated weed management. Here, we discuss the risks to sustainable agriculture from the new resistant crops and present alternatives for research and policy.

3. Norsworthy, J.K., Ward, S.M., Shaw, D.R., Llewellyn, R.S., Nichols, R.L., Webster, T.M., Bradley, K.W., Frisvold, G., Powles, S.B., Burgos, N.R., Witt, W.W., Barrett, M. (2012): Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Science, special issue, pp 31-62

4. Todd A. Gaines, Andrew Cripps, and Stephen B. Powles (2012): Evolved Resistance to Glyphosate in Junglerice (Echinochloa colona) from the Tropical Ord River Region in Australia. Weed Technology 26(3):480-484.

The objective of this study was to determine whether a junglerice population from the tropical Ord River region of northwest Australia was glyphosate resistant, and whether alternative herbicides labeled for junglerice control were still effective. Seed samples collected from the field site were initially screened with glyphosate in the glasshouse, and surviving individuals were self-pollinated for subsequent glyphosate dose-response studies. Glyphosate resistance was confirmed, as the suspected resistant population was found to be 8.6-fold more resistant to glyphosate than a susceptible population based on survival (LD50 of 3.72 kg ha−1), and 5.6-fold more resistant based on biomass reduction (GR50 of 1.16 kg ha−1). The glyphosate-resistant population was susceptible to label-recommended doses of all other herbicides assessed, including three acetyl-CoA carboxylase (ACC) inhibiting herbicides (fluazifop-P, haloxyfop, and sethoxydim), two acetolactate synthase (ALS) inhibiting herbicides (imazamox and sulfometuron), paraquat, and glufosinate. Glyphosate resistance has previously evolved in numerous species found in glyphosate-resistant cropping systems, no-till chemical fallow, fence line, and perennial crop situations. Here we report the evolution of glyphosate resistance in a cropping system that included annual tillage. The evolution of glyphosate resistance in junglerice from a tropical cropping system further demonstrates the need for improved glyphosate stewardship practices globally.

5. Technical Announcement: Widely Used Herbicide Commonly Found in Rain and Streams in the Mississippi River Basin, U.S. Department of the Interior, U.S. Geological Survey

Released: 8/29/2011 8:19:35 AM.

http://www.usgs.gov/newsroom/article.asp?ID=2909

6. A.J. Price, K.S. Balkcom, S.A. Culpepper, J.A. Kelton, R.L. Nichols and H. Schomberg (2011): Glyphosate-resistant Palmer amaranth: A threat to conservation tillage. Journal of Soil and Water Conservation. Vol. 66(4) 265-275

Conservation tillage reduces the physical movement of soil to the minimum required for crop establishment and production. When consistently practiced as a soil and crop management system, it greatly reduces soil erosion and is recognized for the potential to improve soil quality and water conservation and plant available water. Adoption of conservation tillage increased dramatically with the advent of transgenic, glyphosate-resistant crops that permitted in-season, over-the-top use of glyphosate (N-[phosphonomethyl] glycine), a broad-spectrum herbicide with very low mammalian toxicity and minimal potential for off-site movement in soil or water. Glyphosate-resistant crops are currently grown on approximately 70 million ha (173 million ac) worldwide. The United States has the most hectares (45 million ha [99 million ac]) of transgenic, glyphosate-resistant cultivars and the greatest number of hectares (46 million ha [114 million ac]) in conservation tillage. The practice of conservation tillage is now threatened by the emergence and rapid spread of glyphosate-resistant Palmer amaranth (Amaranthus palmeri [S.] Wats.), one of several amaranths commonly called pigweeds. First identified in Georgia, it now has been reported in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee. Another closely related dioecious amaranth, or pigweed, common waterhemp (Amaranthus rudis Sauer), has also developed resistance to glyphosate in Illinois, Iowa, Minnesota, and Missouri. Hundreds of thousands of conservation tillage hectares, some currently under USDA Natural Resources Conservation Service conservation program contracts, are at risk of being converted to higher-intensity tillage systems due to the inability to control these glyphosate-resistant Amaranthus species in conservation tillage systems using traditional technologies. The decline of conservation tillage is inevitable without the development and rapid adoption of integrated, effective weed control strategies. Traditional and alternative weed control strategies, such as the utilization of crop and herbicide rotation and integration of high residue cereal cover crops, are necessary in order to sustain conservation tillage practices

7. Herbicide Resistant Weeds Summary Table. July 26, 2010

Engineered glyphosate resistance is the most widely adopted genetically modified trait in agriculture, gaining widespread acceptance by providing a simple robust weed control system. However, extensive and sustained use of glyphosate as a sole weed control mechanism has led to field selection for glyphosate-resistant weeds and has induced significant population shifts to weeds with inherent tolerance to glyphosate. Additional weed control mechanisms that can complement glyphosate-resistant crops are, therefore, urgently needed. 2,4-dichlorophenoxyacetic acid (2,4-D) is an effective low-cost, broad-spectrum herbicide that controls many of the weeds developing resistance to glyphosate. We investigated the substrate preferences of bacterial aryloxyalkanoate dioxygenase enzymes (AADs) that can effectively degrade 2,4-D and have found that some members of this class can act on other widely used herbicides in addition to their activity on 2,4-D. AAD-1 cleaves the aryloxyphenoxypropionate family of grass-active herbicides, and AAD-12 acts on pyridyloxyacetate auxin herbicides such as triclopyr and fluroxypyr. Maize plants transformed with an AAD-1 gene showed robust crop resistance to aryloxyphenoxypropionate herbicides over four generations and were also not injured by 2,4-D applications at any growth stage. Arabidopsis plants expressing AAD-12 were resistant to 2,4-D as well as triclopyr and fluroxypyr, and transgenic soybean plants expressing AAD-12 maintained field resistance to 2,4-D over five generations. These results show that single AAD transgenes can provide simultaneous resistance to a broad repertoire of agronomically important classes of herbicides, including 2,4-D, with utility in both monocot and dicot crops. These transgenes can help preserve the productivity and environmental benefits of herbicide-resistant crops.

www.weedscience.org

8. S.B. Powles (2010): "Gene amplification delivers glyphosate-resistant weed evolution," Proceedings of the National Academy of Sciences 107 (3): 955-56.

(NO ABSTRACT) Clearly, nature can and will evolve in response to modern agricultural practices. Recurrent selection with herbicides at sublethal rates, including glyphosate, can lead to rapid resistance evolution. Gene amplification can now be added to the category of potential evolutionary paths by which weeds can combat human attempts to control them. The importance of the report by Gaines et al. (2) is that dramatic gene amplification can occur as an evolutionary response to herbicide selection pressure. Aside from contribution to our understanding of evolution this example may reveal novel molecular genetic processes in plants that could be manipulated for targeted gene amplification in genetic engineering. With this development, we have an even stronger basis to urge world agriculture to use glyphosate-resistant crop technology more wisely than has occurred until now. Indeed, the precious herbicide glyphosate is at risk for being driven into redundancy because of overuse without diversity in weed control practices. It is not an exaggeration to state that the potential loss of glyphosate to significant areas of world cropping is a threat to global food production. To avert this situation requires that glyphosate be used more judiciously and with more diversity than is currently the case.

9. Todd A Gaines, Wenli Zhang, Dafu Wang, Bekir Bukun, Stephen T Chisholm, Dale L Shaner, Scott J Nissen, William L Patzoldt, Patrick J Tranel, A Stanley Culpepper, Timothy L Grey, Theodore M Webster, William K Vencill, R Douglas Sammons, Jiming Jiang, Christopher Preston, Jan E Leach and Philip Westra (2010): Gene Amplification confers glyphosate resistance in Amaranthus palmeri. Proceedings of the National Academy of Sciences, Vol. 107 (3), 1029-1034

The herbicide glyphosate became widely used in the United States and other parts of the world after the commercialization of glyphosate-resistant crops. These crops have constitutive overexpression of a glyphosate-insensitive form of the herbicide target site gene, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Increased use of glyphosate over multiple years imposes selective genetic pressure on weed populations. We investigated recently discovered glyphosate-resistant Amaranthus palmeri populations from Georgia, in comparison with normally sensitive populations. EPSPS enzyme activity from resistant and susceptible plants was equally inhibited by glyphosate, which led us to use quantitative PCR to measure relative copy numbers of the EPSPS gene. Genomes of resistant plants contained from 5-fold to more than 160-fold more copies of the EPSPS gene than did genomes of susceptible plants. Quantitative RT-PCR on cDNA revealed that EPSPS expression was positively correlated with genomic EPSPS relative copy number. Immunoblot analyses showed that increased EPSPS protein level also correlated with EPSPS genomic copy number. EPSPS gene amplification was heritable, correlated with resistance in pseudo-F2 populations, and is proposed to be the molecular basis of glyphosate resistance. FISH revealed that EPSPS genes were present on every chromosome and, therefore, gene amplification was likely not caused by unequal chromosome crossing over. This occurrence of gene amplification as an herbicide resistance mechanism in a naturally occurring weed population is particularly significant because it could threaten the sustainable use of glyphosate-resistant crop technology.

10. Waltz, E. (2010): Glyphosate resistance threatens Roundup hegemony. Nature Biotechnology 28, 537538

Weeds are becoming increasingly resistant to glyphosate, a report from the US National Academy of Sciences (NAS) released in April has found. The driving force, according to the report, is farmers' dependence on the weed killer.

11. Binimelis, R,, Pengue, W., Monterroso, I. (2009): Transgenic treadmill: Responses to the emergence and spread of glyphosate-resistant johnsongrass in Argentina. Geoforum 40, 623633.

The broad-spectrum herbicide glyphosate has become the largest-selling crop-protection product worldwide. The increased use of glyphosate is associated with the appearance of a growing number of tolerant or resistant weeds, with socio-environmental consequences apart from the loss of productivity. In 2002, a glyphosate-resistant biotype of johnsongrass (Sorghum halepense (L.)) appeared in Argentina and now covers at least 10,000 ha. This paper analyzes the driving forces behind the emergence and spread of this weed and also examines management responses and their implications. Preventive strategies against glyphosate-resistant johnsongrass fail because of the institutional setting. Reactive measures, however, transfer the risks to the society and the environment through the introduction of novel genetically modified crops that allow the use of yet more herbicide. This in turn reinforces the emergence of herbicide-resistant weeds, constituting a new phenomenon of intensification, the "transgenic treadmill”.

12. Bindraban, P.S., Franke. A.C. Ferrar, D.O., Ghersa, C.M., Lotz, L.A.P., Nepomuceno, A., Smulders, M.J.M., van de Wiel, C.C.M. (2009): GM-related sustainability: agroecological impacts, risks and opportunities of soy production in Argentina and Brazil, Plant Research International, Wageningen UR, Netherlands, Report 259.

http://gmsoydebate.global-connections.nl/sites/gmsoydebate.globalconnections.nl/files/library/2009%20WUR%20Research%20Report%20GM%20Soy.pdf

13. Knispel A L, McLachlan S M, Van Acker R, Friesen L F. (2008): Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science 56, 7280.

Gene flow among herbicide-resistant (HR) canola varieties can lead to the development of multiple HR canola plants, creating volunteer canola management challenges for producers. In western Canada, escaped populations of HR canola are ubiquitous outside of cultivated fields, yet the extent of gene flow resulting in herbicide resistance trait stacking in individuals within these populations remains unknown. The objectives of this study were to document the resence of single and multiple herbicide resistance traits and assess the extent of gene flow within escaped canola populations. Seed was collected from 16 escaped canola populations along the verges of fields and roadways in four agricultural regions in southern Manitoba from 2004 to 2006. Glyphosate resistance was found in 14 (88%) of these populations, glufosinate resistance in 13 (81%) populations, and imidazolinone resistance in five (31%) populations. Multiple herbicide resistance was observed at levels consistent with previously published canola outcrossing rates in 10 (62%) of the tested populations. In 2005 and 2006, maternal plants from two escaped populations were tested using trait indicator test strips for glyphosate and glufosinate resistance to confirm outcrossing events. In 2005, two of 13 tested maternal plants with single herbicide resistance traits produced progeny with both glyphosate and glufosinate resistance. In 2006, of 21 tested plants, 10 single HR maternal plants produced multiple HR progeny, and five nonresistant maternal plants produced resistant offspring. This is the first report indicating that intraspecific gene flow results in stacking of herbicide resistance traits in individuals within escaped canola populations, confirming that multiple HR canola volunteers are not confined to agricultural fields. Results of this study suggest that escaped populations of crop plants can contribute to the spread of genetically engineered novel traits, which has important implications for containment, especially for highly controversial pharmaceutical and industrial traits in crop plants.

14. Rahman, A., James, T.K., Trolove, M.R. (2008): Chemical control options for the dicamba resistant biotype of fathen (Chenopodium album). New Zealand Plant Protection 61, 287291.

Three field trials were conducted over two growing seasons to evaluate the efficacy of potential selective post-emergence herbicides against dicamba resistant fathen (Chenopodium album). Results confirmed the presence of the biotype, which tolerated dicamba upto 2400 g/ha, eight times the recommended field rate. Post emergence applications bromoxynil, pyridate, nicosulfuron and mesotrione all showed equally good efficacy on both susceptible and resistant biotypes. Flumestulam provided good control in one of three trials. Nicosulfuron and mesotrione provided long term residual control in all trials, with nicosulfuron also being more effective on grass weeds. The 2400 g/ ha rate of dicamba severely damaged the maize crop, resulting in increased weed cover and reduced grain yield. Numbers of viable fathen seeds in the soil at the end of the growing season remained similar to those recorded before planting, except in plots that provided good control of fathen, where numbers had dropped considerably.

15. Vila-Aiub M.M., Vidal A R, Balbi M.C, Gundel P.E, Trucco F, and Ghersa C.M. (2007): Glyphosate-resistant weeds of South American cropping systems: an overview. Pest Management Science, 64, 366371.

Herbicide resistance is an evolutionary event resulting from intense herbicide selection over genetically diverse weed populations. In South America, orchard, cereal and legume cropping systems show a strong dependence on glyphosate to control weeds. The goal of this report is to review the current knowledge on cases of evolved glyphosate-resistant weeds in South American agriculture. The first reports of glyphosate resistance include populations of highly diverse taxa (Lolium multiflorum Lam., Conyza bonariensis L., C. canadensis L.). In all instances, resistance evolution followed intense glyphosate use in fruit fields of Chile and Brazil. In fruit orchards from Colombia, Parthenium hysterophorus L. has shown the ability to withstand high glyphosate rates. The recent appearance of glyphosate-resistant Sorghum halepense L. and Euphorbia heterophylla L. in glyphosate-resistant soybean fields of Argentina and Brazil, respectively, is of major concern. The evolution of glyphosate resistance has clearly taken place in those agroecosystems where glyphosate exerts a strong and continuous selection pressure on weeds. The massive adoption of no-till practices together with the utilization of glyphosate-resistant soybean crops are factors encouraging increase in glyphosate use. This phenomenon has been more evident in Argentina and Brazil. The exclusive reliance on glyphosate as the main tool for weed management results in agroecosystems biologically more prone to glyphosate resistance evolution.

16. Vidal A.R, Trezzi M.M, Prado R, Ruiz-Santaella J.P, and Vila-Aiub M. (2007): Glyphosate resistant biotypes of wild poinsettia (Euphorbia heterophylla L.) and its risk analysis on glyphosate-tolerant soybeans. Journal of Food, Agriculture & Environment 5: 265269.

The continuous use of a single herbicide for weed control can result in selection of biotypes resistant to that compound. Greenhouse experiments were conducted to assess the occurrence of wild poinsettia (Euphorbia heterophylla, EPHHL) resistant biotypes to glyphosate. Two suspected glyphosate- resistant biotypes from the northern part of Rio Grande do Sul, Brazil, were compared to known glyphosate-susceptible biotypes. Dose-response curves were used to compare the biotypes, with rates ranging from 0 to 450 g ha-1 in one experiment, and from 0 to 1200 g ha-1 in another. The resistance factor, calculated with the I50 data, indicated the resistant biotypes were about three times less sensitive to glyphosate than the susceptible biotypes. This is the first report of a glyphosate-resistant biotype in a weed species of major importance and distribution in Brazil. A risk analysis is discussed for the occurrence of glyphosate-resistant wild poinsettia in glyphosate-tolerant soybeans.

17. Sandermann H. (2006): Plant biotechnology: ecological case studies on herbicide resistance. Trends in Plant Science 11 (7), 324328.

The emerging field of molecular ecology aims to improve the ecological predictability of transgenic crop plants. The most widely cultivated lines are Roundup- Readyw plants, which are genetically modified to be resistant to the broad-spectrum herbicide glyphosate. Recent publications demonstrate two ecological effects that were not anticipated: the widespread emergence of glyphosate-resistant weed biotypes and the formation of a metabolic herbicidal residue. Both effects appear to be due to the increased use of glyphosate rather than the genetic modification in the transgenic crop plant. With one prominent exception, opinions collected from the literature point towards a certain degree of resistance mismanagement and an inadequate testing of the ecological effects of extensive glyphosate use.

18. Owen M D K and Zelaya I A (2005): Herbicide-resistant crops and weed resistance to herbicides. Pest Manag. Sci. 61: 301-311.

The adoption of genetically modified (GM) crops has increased dramatically during the last 3 years, and currently over 52 million hectares of GM crops are planted world-wide. pproximately 41 million hectares of GM crops planted are herbicide-resistant crops, which includes an estimated 33.3million hectares of herbicide-resistant soybean. Herbicide-resistant maize, canola, cotton and soybean accounted for 77% of the GM crop hectares in 2001. However, sugarbeet, wheat, and as many as 14 other crops have transgenic herbicide-resistant cultivars that may be commercially available in the near future. There are many risks associated with the production of GM and herbicide-resistant crops, including problems with grain contamination, segregation and introgression of herbicide-resistant traits, marketplace acceptance and an increased reliance on herbicides for weed control. The latter issue is represented in the occurrence of weed population shifts, the evolution of herbicide-resistant weed populations and herbicide-resistant crops becoming volunteer weeds. Another issue is the ecological impact that simple weed management programs based on herbicide-resistant crops have on weed communities. Asiatic dayflower (Commelina cumminus L) common lambsquarters (Chenopodium album L) and wild buckwheat (Polygonum convolvulus L) are reported to be increasing in prominence in some agroecosystems due to the simple and significant selection pressure brought to bear by herbicideresistant crops and the concomitant use of the herbicide. Finally, evolution of herbicide-resistant weed populations attributable to the herbicide-resistant crop/herbicide program has been observed. Examples of herbicide-resistant weeds include populations of horseweed (Conyza canadensis (L) Cronq) resistant to N-(phosphonomethyl)glycine (glyphosate). An important question is whether or not these problems represent significant economic issues for future agriculture.

Benbrook C.M. (2005): Rust, resistance, run down soils, and rising costs Problems facing soybean producers in Argentina. AgBioTech InfoNet, Technical Paper No 8, January.

19. Nandula V.K., Reddy, K., Duke, S. (2005): Glyphosate-resistant weeds: Current status and future outlook. Outlooks on Pest Management 16, 183187.

Introduction of glyphosate-resistant transgenic crops has revolutionized weed management. GR crops as weed management tools have allowed farmers to manage weeds more effectively and economically. High levels of adoption of GR crops by U.S. farmers have dramatically increased the use of glyphosate, with a concomitant decrease in use of other herbicides. This has impacted weed communities. Evolution of weeds resistant to glyphosate and weed species shifts towards naturally resistant species in GR crops require alternative strategies to manage weeds. GR crops should not be relied solely on their respective herbicides to the exclusion of other weed control methods, and should be used within integrated management systems. The problem of GR weeds is real, and farmers have to understand that continuous use of glyphosate without alternative strategies will likely result in evolution of more GR weeds. Even in the short term, no one can predict the future loss of glyphosate efficacy due to weed species shifts and evolution of glyphosate resistance. This will depend on whether mitigation strategies will be adopted by farmers and the inherent predisposition of many different weed species to evolve resistance or to move into agricultural ecosystems.

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20. Vitta J I, Tuesca D, Puricelli E. (2004): Widespread use of glyphosate tolerant soybean and weed community richness in Argentina. Agriculture, Ecosystems & Environment 103, 3621624.

Trends in weed richness responses to widespread use of glyphosate tolerant soybean in Argentina are presented. An experiment was carried out since 1997 to analyse weed community changes associated with the continuous and exclusive use of glyphosate. Sampling results showed that weed richness in soybean decreased or remained stable early in the season before glyphosate application but increased at harvest. A survey of crop advisers indicated that 37 species were of increasing, 18 species of decreasing importance. It is suggested that successional changes are taking place in the cropping systems in response to changes in weed control practices.

21. Branford S. (2004): Argentina's bitter harvest. New Scientist, 17 April

When genetically modified soya came on the scene it seemed like a heaven-sent solution to Argentina's agricultural problems. Now soya is being blamed for an environmental crisis that is threatening the country's fragile economic recovery. Sue Branford discovers how it all went wrong.

22. Kilman, S. 2010. Superweed outbreak triggers arms race. Wall Street Journal, 4 June.

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24. Osunsami, S. 2009. Killer pig weeds threaten crops in the South. ABC World News, 6 October.

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27. Johnson, B. and Davis, V. 2005. Glyphosate resistant horseweed (marestail) found in 9 more Indiana counties. Pest & Crop, May 13.

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28. Randerson, J. 2002. Genetically-modified superweeds "not uncommon". New Scientist, 05 February.

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PEST DYNAMICS INCL. RESISTANCE

1. Hagenbucher S, Wackers FL, Wettstein FE, Olson DM, Ruberson JR, Romeis J. (2013): Pest tradeoffs in technology: reduced damage by caterpillars in Bt cotton benefits aphids. Proc R Soc B 20130042.

The rapid adoption of genetically engineered (GE) plants that express insecticidal Cry proteins derived from Bacillus thuringiensis (Bt) has raised concerns about their potential impact on non-target organisms. This includes the possibility that non-target herbivores develop into pests. Although studies have now reported increased populations of non-target herbivores in Bt cotton, the underlying mechanisms are not fully understood. We propose that lack of herbivore-induced secondary metabolites in Bt cotton represents a mechanism that benefits non-target herbivores. We show that, because of effective suppression of Bt-sensitive lepidopteran herbivores, Bt cotton contains reduced levels of induced terpenoids. We also show that changes in the overall level of these defensive secondary metabolites are associated with improved performance of a Bt-insensitive herbivore, the cotton aphid, under glasshouse conditions. These effects, however, were not as clearly evident under field conditions as aphid populations were not correlated with the amount of terpenoids measured in the plants. Nevertheless, increased aphid numbers were visible in Bt cotton compared with non-Bt cotton on some sampling dates. Identification of this mechanism increases our understanding of how insect-resistant crops impact herbivore communities and helps underpin the sustainable use of GE varieties.

http://dx.doi.org/10.1098/rspb.2013.0042

2. András Székács and Béla Darvas (2012): Chapter 10: Comparative Aspects of Cry Toxin Usage in Insect Control. In "Advanced Technologies for Managing Insect Pests". Eds. Isaac Ishaaya, Subba Reddy Palli and A Rami Horowitz. Springer Science + Business Media. 195-230.

Conclusion: Based on the above, Bt -based bioinsecticides and crops cannot be considered by far as equivalent technologies. Their application differs as Bt bioinsecticides allow singular applications, while Bt crops exert a continuous production of the Cry toxin. This results in higher environmental doses of the plant-expressed toxin(s) than in the case of the Bt bioinsecticide. For example a single treatment of Dipel bioinsecticide at the registered dosage (1 kg/ha) contains 4.860.2 mg/ha (average 20.6 mg/ha) of bioavailable Cry1Ab toxin, while the amount of bioaccessible amount of Cry1Ab toxin is 0.0858.16 g/ha. In contrast, the production of plant-expressed Cry1Ab toxin was found to be 147456 g Cry1Ab toxin/ha, representing 1856 treatments with Dipel (on the basis of its maximally detected bioaccessible Cry1Ab toxin content, 8.16 g/ha). The level of plant-expressed Cry1Ab toxin can be further elevated by soil fertilization (2.36.8-fold) and the use of long maturation maize varieties (2.55.8-fold), representing, in worst case scenarios, in 6251,930 treatments with Dipel. Moreover, it has to be mentioned that stacked genetic events may further elevate toxin production (twofold). These ratios are even higher if lower bioaccessible Cry1Ab protoxin content biopesticides or bioavailable Cry1Ab toxin contents are considered. Beside toxin ratios, another characteristic difference is that while Bt bioinsecticides are composed of several crystalline toxins, single genetic event Bt crops express only a single toxin molecule. This has severe consequences in resistance development, which may be alleviated, yet not eliminated by the use of "pyramid" Bt event varieties, expressing several Cry toxins acting on the same insect order, as the evolutionary driving force remain the same. The active ingredient of Bt bioinsecticides are bacterial protoxins stabilized in crystalline form and requiring enzymatic activation, while Bt plants (e.g., MON 810) express a truncated form of the protoxin, so-called preactivated toxin. This has severe consequences in product registration, as the active ingredient toxin in the Bt crop is not the registered active substance of the corresponding Bt bioinsecticide, and the required toxicology studies have been carried out not with the plant-expressed preactivated toxin, but with the bacterial protoxin or the enzyme-activated active toxin. Moreover, commercial ELISA systems utilizing antibodies against the bacterial protoxin and analytical standards of that protoxin consistently underdetect actual toxin content in Bt plants due to their lower cross-reactivities to the plant-expressed preactivated toxin. As a result, all reported results obtained by protoxin-based ELISAs, including manufacturer documentation, are subject to correction. And finally, although Bt crops have been widely advocated to be included in integrated pest management (IPM) practices or even in ecological agriculture, Bt crops cannot fulfill the main ecological principle of IPM that any protection measures should be timed only to the period(s) when pest damage exceeds the critical level, and therefore, regardless how environmentally mild their active ingredient is, do not comply with IPM.

3. Haonan Zhang, Wen Tian Jing Zhao, Lin Jin Jun, Yang Chunhui, Liu Yihua, Yang Shuwen, Wu Kongming, Wu Jinjie Cui, Bruce E. Tabashnik and Yidong Wu (2012): Diverse genetic basis of field-evolved resistance to Bt cotton in cotton bollworm from China. Proceedings of the National Academy of Sciences, vol. 109, issue 26, pp. 10275-10280

Evolution of pest resistance reduces the efficacy of insecticidal proteins from Bacillus thuringiensis (Bt) used in sprays or in transgenic crops. Although several pests have evolved resistance to Bt crops in the field, information about the genetic basis of field-evolved resistance to Bt crops has been limited. In particular, laboratory-selected resistance to Bt toxin Cry1Ac based on recessive mutations in a gene encoding a toxin-binding cadherin protein has been identified in three major cotton pests, but previous work has not determined if such mutations are associated with field-selected resistance to Bt cotton. Here we show that the most common resistance alleles in field populations of cotton bollworm, Helicoverpa armigera, selected with Bt cotton in northern China, had recessive cadherin mutations, including the deletion mutation identified via laboratory selection. However, unlike all previously studied cadherin resistance alleles, one field-selected cadherin resistance allele conferred nonrecessive resistance. We also detected nonrecessive resistance that was not genetically linked with the cadherin locus. In field-selected populations, recessive cadherin alleles accounted for 7584% of resistance alleles detected. However, most resistance alleles occurred in heterozygotes and 5994% of resistant individuals carried at least one nonrecessive resistance allele. The results suggest that resistance management strategies must account for diverse resistance alleles in field-selected populations, including nonrecessive alleles.

4. Gassmann AJ, Petzold-Maxwell JL, Keweshan RS and Dunbar MW (2011): Field evolved resistance to Bt maize by Western Corn Rootworm. PLoS One 6(7): 222629

Crops engineered to produce insecticidal toxins derived from the bacterium Bacillus thuringiensis (Bt) are planted on millions of hectares annually, reducing the use of conventional insecticides and suppressing pests. However, the evolution of resistance could cut short these benefits. A primary pest targeted by Bt maize in the United States is the western corn rootworm Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae).This is the first report of field-evolved resistance to a Bt toxin by the western corn rootworm and by any species of Coleoptera. Insufficient planting of refuges and non-recessive inheritance of resistance may have contributed to resistance. These results suggest that improvements in resistance management and a more integrated approach to the use of Bt crops may be necessary.

5. Jennifer H. Zhao, Peter Ho, Hossein Azadi (2011): Benefits of Bt cotton counterbalanced by secondary pests? Perceptions of ecological change in China. Environmental Monitoring and Assessment. Volume 173, Issue 1-4, pp 985-994

In the past, scientific research has predicted a decrease in the effectiveness of Bt cotton due to the rise of secondary and other sucking pests. It is suspected that once the primary pest is brought under control, secondary pests have a chance to emerge due to the lower pesticide applications in Bt cotton cultivars. Studies on this phenomenon are scarce. This article furnishes empirical evidence that farmers in China perceive a substantial increase in secondary pests after the introduction of Bt cotton. The research is based on a survey of 1,000 randomly selected farm households in five provinces in China. We found that the reduction in pesticide use in Bt cotton cultivars is significantly lower than that reported in research elsewhere. This is consistent with the hypothesis suggested by recent studies that more pesticide sprayings are needed over time to control emerging secondary pests, such as aphids, spider mites, and lygus bugs. Apart from farmers' perceptions of secondary pests, we also assessed their basic knowledge of Bt cotton and their perceptions of Bt cotton in terms of its strengths and shortcomings (e.g., effectiveness, productivity, price, and pesticide use) in comparison with non-transgenic cotton.

An erratum to this article can be found at http://dx.doi.org/10.1007/s10661-012-2699-5

6. Dhurua S and Gujar GT (2011): Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) from India. Pest Management Science. Vol. 67 (8): 898-903.

The pink bollworm is one of the most destructive pests of cotton. Transgenic cotton producing Bt toxin Cry1Ac or a combination of Cry1Ac and Cry2Ab2 has been used effectively against this pest. However, some other insects have evolved resistance to Bt toxins in the field. During the 20072008 and 20082009 seasons, pink bollworm populations in India were surveyed to evaluate their responses to Cry1Ac and seed powder containing Cry1Ac and Cry2Ab2. The results provide evidence that resistance to Cry1Ac had evolved by 2008 in a population sampled from non-Bt cotton in the Amreli district of Gujarat in western India. The median lethal concentration of Cry1Ac for five-day-old larvae (LC50) was significantly higher for insects derived in 2008 from Amreli than for any of the other field populations tested from four locations in India. For Cry1Ac, the mean LC50 for the strain derived from Amreli in 2008 was 44 times higher than for the most susceptible population. However, for seed powder of Bollgard II containing primarily Cry2Ab2, the 2008 Amreli population was only slightly less susceptible than the most susceptible population. The data reported here constitute the first evidence of field-evolved resistance of pink bollworm to Cry1Ac. This initial evidence spurred more extensive evaluations during the 20092010 growing season, which confirmed field-evolved resistance to Cry1Ac in Amreli. The lack of cross-resistance to Cry2Ab2 suggests that plants producing this toxin are likely to be more effective against resistant populations than plants producing only Cry1Ac.

7. Storer NP, Babcock JM, Schlenz M, Meade T, Thompson GD, Bing JW and Huckaba RM (2010): Discovery and characterization of field resistance to Bt maize: Spodoptera frugiperda (Lepidoptera: Noctuidae) in Puerto Rico. J Econ Entomol.103(4): 1031-8.

Transgenic maize, Zea mays L., event TC1507 produces the Cry1F protein to provide protection from feeding by several important lepidopteran pests, including Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae). Reports of reduced field performance against this species in Puerto Rico were investigated, and laboratory bioassays showed that S. frugiperda collected from the affected area exhibited lower sensitivity to the Cry1F protein compared with typical colonies from other regions. The resistance was shown to be autosomally inherited and highly recessive. The Puerto Rico colony was shown to be moderately less sensitive than susceptible laboratory strains to Cry1Ab and Cry1Ac, but the differences in sensitivity were dramatically smaller than for Cry1F. Potential contributory factors to the emergence of resistance to Cry1F in Puerto Rico populations of S. frugiperda include the tropical island geography, unusually large population sizes in 2006, and drought conditions reducing the availability of alternative hosts. In response to this resistance incident, the technology providers have stopped commercial sales of TC1507 maize in Puerto Rico pending potential reversion to susceptibility.

8. Downes S, Parker T and Mahon R (2010): Incipient resistance of Helicoperva punctigera to the Cry2Ab Bt toxin in Bollgard II cotton. PLoS One 5(9): e12567.

Combinations of dissimilar insecticidal proteins (“pyramids”) within transgenic plants are predicted to delay the evolution of pest resistance for significantly longer than crops expressing a single transgene. Field-evolved resistance to Bacillus thuringiensis (Bt) transgenic crops has been reported for first generation, single-toxin varieties and the Cry1 class of proteins. Our five year data set shows a significant exponential increase in the frequency of alleles conferring Cry2Ab resistance in Australian field populations of Helicoverpa punctigera since the adoption of a second generation, two-toxin Bt cotton expressing this insecticidal protein. Furthermore, the frequency of cry2Ab resistance alleles in populations from cropping areas is 8-fold higher than that found for populations from non-cropping regions. This report of field evolved resistance to a protein in a dual-toxin Bt-crop has precisely fulfilled the intended function of monitoring for resistance; namely, to provide an early warning of increases in frequencies that may lead to potential failures of the transgenic technology. Furthermore, it demonstrates that pyramids are not 'bullet proof' and that rapid evolution to Bt toxins in the Cry2 class is possible.

9. Caccia S, Hernández-Rodríguez CS, Mahon RJ, Downes S, James W, et al. (2010): Binding Site Alteration Is Responsible for Field-Isolated Resistance to Bacillus thuringiensis Cry2A Insecticidal Proteins in Two Helicoverpa Species. PLoS ONE 5(4): e9975

Evolution of resistance by target pests is the main threat to the long-term efficacy of crops expressing Bacillus thuringiensis (Bt) insecticidal proteins. Cry2 proteins play a pivotal role in current Bt spray formulations and transgenic crops and they complement Cry1A proteins because of their different mode of action. Their presence is critical in the control of those lepidopteran species, such as Helicoverpa spp., which are not highly susceptible to Cry1A proteins. In Australia, a transgenic variety of cotton expressing Cry1Ac and Cry2Ab (Bollgard II) comprises at least 80% of the total cotton area. Prior to the widespread adoption of Bollgard II, the frequency of alleles conferring resistance to Cry2Ab in field populations of Helicoverpa armigera and Helicoverpa punctigera was significantly higher than anticipated. Colonies established from survivors of F2 screens against Cry2Ab are highly resistant to this toxin, but susceptible to Cry1Ac.

10. Ranjith MT, Prabhuraj A and Srinivasa YB (2010): Survival and reproduction of natural populations of Helicoverpa armigera on Bt-cotton hybrids in Raichur, India. Current Science, Vol. 99 (11).

Transgenic Bt-cotton is commercially cultivated on the rationale that it produces toxins that defend the plants primarily from caterpillars damaging cotton bolls. From the context of crop protection, it is important that these bollworms remain susceptible to the toxins, so that their populations are under check. However, if certain individuals are able to survive and breed on the transgenics, they can build populations resistant to the toxins. In one such instance we discovered individuals of Helicoverpa armigera, the most prominent among bollworms in India, surviving on commercial Bt-cotton hybrids containing single (Cry1Ac) and double (Cry1Ac and Cry2Ab) genes in experimental plots of the University of Agricultural Sciences, Raichur campus, India. Analyses of various biological parameters measured through laboratory breeding on the respective hybrids revealed that these surviving individuals could not only complete their life cycle but also reproduce. A proportion of individuals of the succeeding generation were also able to complete their life cycle on the transgenic commercial hybrids. Interestingly, many of the biological parameters of the bollworm across Bt and non-Bt hybrids were mostly comparable. These results not only validate the occurrence of natural populations of H. armigera on Btcotton hybrids, but also provide evidence for its survival and successful reproduction in India.

11. Carriere Y, Crowder DW and Tabashnik BE (2010): Evolutionary ecology of insect adaptation to Bt crops. Evolutionary Applications (Special Issue: Evolution in Agro- Ecosystems) Vol. 3, Issue 5-6: 561-73.

Transgenic crops producing Bacillus thuringiensis (Bt) toxins are used worldwide to control major pests of corn and cotton. Development of strategies to delay the evolution of pest resistance to Bt crops requires an understanding of factors affecting responses to natural selection, which include variation in survival on Bt crops, heritability of resistance, and fitness advantages associated with resistance mutations. The two main strategies adopted for delaying resistance are the refuge and pyramid strategies. Both can reduce heritability of resistance, but pyramids can also delay resistance by reducing genetic variation for resistance. Seasonal declines in the concentration of Bt toxins in transgenic cultivars, however, can increase the heritability of resistance. The fitness advantages associated with resistance mutations can be reduced by agronomic practices, including increasing refuge size, manipulating refuges to increase fitness costs, and manipulating Bt cultivars to reduce fitness of resistant individuals. Manipulating costs and fitness of resistant individuals on transgenic insecticidal crops may be especially important for thwarting evolution of resistance in haplodiploid and parthenogenetic pests. Field-evolved resistance to Bt crops in only five pests during the last 14 years suggests that the refuge strategy has successfully delayed resistance, but the accumulation of resistant pests could accelerate.

12. Kruger M, Van Rensburg JBJ and Van den Berg J (2009): Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. Crop Protection 28 (8): 684-9.

Bt maize has been grown at the Vaalharts irrigation scheme in South Africa since its first release during 1998. Interest in Bt maize refuge compliance, pest incidence and production practices at Vaalharts were recently stimulated by the first report of field resistance of Busseola fusca (Lepidoptera: Noctuidae) to Bt maize. Objectives of this study were to evaluate farmer's perceptions of the regulatory aspects guiding the planting of Bt maize and refugia and how the field situation developed between 1998 and 2008. A survey, using a self-administered questionnaire, was conducted amongst 80 farmers at the irrigation scheme. The questionnaire addressed signing of contracts upon purchasing genetically modified (GM) seed, refuge compliance, refuge design and general farming practices. Farmers were also questioned on the perceived benefits and disadvantages of Bt maize and their perceptions of the pest status of B. fusca. The two greatest advantages associated with Bt maize were indicated to be convenient management (88%) and increased productivity (61.3%) while 42.5% indicated that they perceived Bt-technology to be environmental friendly. Initial levels of refuge compliance were low, and even though farmers were obligated to plant a refuge area for each Bt maize field, only 7.7% of farmers planted refuges during 1998. This number increased to 100% during 2008. Eight percent of farmers, however, indicated that they did not plant a refuge field for each Bt maize field, which was justified on the basis of small farm sizes (25ha). Nearly all farmers (99.8%) allow no spatial separation between the Bt maize field and adjacent refuge area. Farmers preferred to plant the refuge option where 5% of the field area is planted to conventional maize, which is not sprayed with insecticide instead of the 20% refuge area on which insecticide application against the target pest is allowed.

13. Lovei GL, Andow DA, Arpaia S (2009): Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environ Entomol. 38(2): 293-306

This review uses a data-driven, quantitative method to summarize the published, peer-reviewed literature about the impact of genetically modified (GM) plants on arthropod natural enemies in laboratory experiments. The method is similar to meta-analysis, and, in contrast to a simple author-vote counting method used by several earlier reviews, gives an objective, data-driven summary of existing knowledge about these effects. Significantly more non-neutral responses were observed than expected at random in 75% of the comparisons of natural enemy groups and response classes. These observations indicate that Cry toxins and proteinase inhibitors often have non-neutral effects on natural enemies. This synthesis identifies a continued bias toward studies on a few predator species, especially the green lacewing, Chrysoperla carnea Stephens, which may be more sensitive to GM insecticidal plants (16.8% of the quantified parameter responses were significantly negative) than predators in general (10.9% significantly negative effects without C. carnea). Parasitoids were more susceptible than predators to the effects of both Cry toxins and proteinase inhibitors, with fewer positive effects (18.0%, significant and nonsignificant positive effects combined) than negative ones (66.1%, significant and nonsignificant negative effects combined). GM plants can have a positive effect on natural enemies (4.8% of responses were significantly positive), although significant negative (21.2%) effects were more common. Although there are data on 48 natural enemy species, the database is still far from adequate to predict the effect of a Bt toxin or proteinase inhibitor on natural enemies.

14. Tabashnik B E, Gassmann A J, Crowder D W and Carrière Y. (2008): Insect resistance to Bt crops: Evidence versus Theory. Nature Biotechnology 26: 199-202.

Evolution of insect resistance threatens the continued success of transgenic crops producing Bacillus thuringiensis (Bt) toxins that kill pests. The approach used most widely to delay insect resistance to Bt crops is the refuge strategy, which requires refuges of host plants without Bt toxins near Bt crops to promote survival of susceptible pests. However, large-scale tests of the refuge strategy have been problematic. Analysis of more than a decade of global monitoring data reveals that the frequency of resistance alleles has increased substantially in some field populations of Helicoverpa zea, but not in five other major pests in Australia, China, Spain and the United States. The resistance of H. zea to Bt toxin Cry1Ac in transgenic cotton has not caused widespread crop failures, in part because other tactics augment control of this pest. The field outcomes documented with monitoring data are consistent with the theory underlying the refuge strategy, suggesting that refuges have helped to delay resistance.

15. Meihis LN, Higdon ML, Siegfried BD, Miller NJ, Sappington TW, Ellersieck MR, Spencer TA & Hibbard BE (2008): Increased survival of western corn rootworm on transgenic corn within 3 generations of on-plant greenhouse selection. Proc Natl Acad Sci USA 105(49): 19177-82.

To delay evolution of insect resistance to transgenic crops producing Bacillus thuringiensis (Bt) toxins, nearby "refuges" of host plants not producing Bt toxins are required in many regions. Such refuges are expected to be most effective in slowing resistance when the toxin concentration in Bt crops is high enough to kill all or nearly all insects heterozygous for resistance. However, Bt corn, Zea mays, introduced recently does not meet this "high-dose" criterion for control of western corn rootworm (WCR), Diabrotica virgifera virgifera. A greenhouse method of rearing WCR on transgenic corn expressing the Cry3Bb1 protein was used in which approximately 25% of previously unexposed larvae survived relative to isoline survival (compared to 1-4% in the field). After three generations of full larval rearing on Bt corn (Constant-exposure colony), WCR larval survival was equivalent on Bt corn and isoline corn in greenhouse trials, and the LC(50) was 22-fold greater for the Constant-exposure colony than for the Control colony in diet bioassays with Cry3Bb1 protein on artificial diet. After six generations of greenhouse selection, the ratio of larval recovery on Bt corn to isoline corn in the field was 11.7-fold greater for the Constant-exposure colony than the Control colony. Removal from selection for six generations did not decrease survival on Bt corn in the greenhouse. The results suggest that rapid response to selection is possible in the absence of mating with unexposed beetles, emphasizing the importance of effective refuges for resistance management.

16. Cloutier C, Boudreault S and Michaud D. (2008): Impact of Colorado potato beetle resistant potatoes on non-target arthropods: a meta-analysis of factors potentially involved in the failure of a Bt transgenic plant. Cahiers Agricultures 17 (4): 388-394

The relatively high specificity of transgenic plants based on Cry toxins of Bacillus thuringiensis (Bt) implies the possibility of upward agroecosystemic cascades toward new equilibria among arthropods associating with cultivated plants. We examine the hypothesis that exclusion of the Colorado potato beetle from potato expressing the Cry3a toxin increases the abundance of non-target herbivores, which indirectly favours the abundance of herbivore-dependent predators and omnivores foraging on agricultural plants. We examined the impact of Bt potato on non-target arthropod taxa, based on impact studies conducted during development of the Newleaf ® Bt potato in North America. Of 32 field tests comparing Bt potato to non-transgenic controls, 14 (42%) revealed a significant, positive effect on the abundance of sucking insects (aphids, leafhoppers, mirids, thrips). Among 72 tests on generalist predators that were simultaneously monitored, 14 (~20%) also revealed significant positive effects. Such positive effects on predators can best be explained by their abundance being increased as a result of greater productivity due to overabundance of sucking insect prey, which are selectively favoured by the high specificity of the Cry3a Bt toxin. Our results support the idea that development of the Bt potato may have been hampered in part by its positive effects on sucking insect pests, and underline the importance of conserving the natural enemies of secondary pests that are indirectly favoured.

17. van Rensburg JBJ (2007): First report of field resistance by the stem borer, Busseola fusca (Fuller) to Bt transgenic maize. S. Afr. J. Plant Soil 24(3): 147-151.

This is the first report of field-evolved resistance to a Bt toxin by the western corn rootworm and by any species of Coleoptera. Insufficient planting of refuges and non-recessive inheritance of resistance may have contributed to resistance. These results suggest that improvements in resistance management and a more integrated approach to the use of Bt crops may be necessary.

18. Faria CA, Wäckers FL, Pritchard J, Barrett DA and Turlings TCJ (2007): High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. PLoS ONE 2(7): e600

Concerns about possible undesired environmental effects of transgenic crops have prompted numerous evaluations of such crops. So-called Bt crops receive particular attention because they carry bacteria-derived genes coding for insecticidal proteins that might negatively affect non-target arthropods. Here we show a remarkable positive effect of Bt maize on the performance of the corn leaf aphid Rhopalosiphum maidis, which in turn enhanced the performance of parasitic wasps that feed on aphid honeydew. Within five out of six pairs that were evaluated, transgenic maize lines were significantly more susceptible to aphids than their near-isogenic equivalents, with the remaining pair being equally susceptible. The aphids feed from the phloem sieve element content and analyses of this sap in selected maize lines revealed marginally, but significantly higher amino acid levels in Bt maize, which might partially explain the observed increased aphid performance. Larger colony densities of aphids on Bt plants resulted in an increased production of honeydew that can be used as food by beneficial insects. Indeed, Cotesia marginiventris, a parasitoid of lepidopteran pests, lived longer and parasitized more pest caterpillars in the presence of aphid-infested Bt maize than in the presence of aphid-infested isogenic maize. Hence, depending on aphid pest thresholds, the observed increased susceptibility of Bt maize to aphids may be either a welcome or an undesirable side effect.

19. Fangneng H, Rogers LB and Xiaoyi W (2007): Resistance of sugarcane borer to Bacillus thuringiensis Cry1Ab toxin. Entomologia Experimentalis et Applicata 124(1):117-123.

The sugarcane borer, Diatraea saccharalis (F.) (Lepidoptera: Crambidae), strain (F52-3-R) was developed from F3 survivors of a single-pair mating on commercial Cry1Ab Bacillus thuringiensis (Bt) corn plants in the greenhouse. The susceptibility of a Bt-susceptible and the F52-3-R strain of D. saccharalis to trypsin-activated Cry1Ab toxin was determined in a laboratory bioassay. Neonate-stage larvae were fed a meridic diet incorporating Cry1Ab toxin at a concentration range of 0.0625 to 32 μg g−1. Larval mortality, larval weight, and number of surviving larvae that did not gain significant weight (<0.1 mg per larva) were recorded on the 7th day after inoculation. The F52-3-R strain demonstrated a significant level of resistance to the activated Cry1Ab toxin. Larval mortality of the Bt-susceptible strain increased in response to higher concentrations of Cry1Ab toxin, exceeding 75% at 32 μg g−1, whereas mortality of the F52-3-R strain was below 8% across all Cry1Ab concentrations. Using a measure of practical mortality (larvae either died or gained no weight), the median lethal concentration (LC50) of the F52-3-R strain was 102-fold greater than that of the Bt-susceptible insects. Larval growth of both Bt-susceptible and F52-3-R strains was inhibited on Cry1Ab-treated diet, but the inhibition of the F52-3-R strain was significantly less than that of the Bt-susceptible insects. These results confirm that the survival of the F52-3-R strain on commercial Bt corn plants was related to Cry1Ab protein resistance and suggest that this strain may have considerable value in studying resistance management strategies for Bt corn.

20. Wang, S., Just, D. R. and Pinstrup-Andersen, P. (2006): 'Tarnishing Silver Bullets: BtTechnology Adoption, Bounded Rationality and the Outbreak of Secondary Pest Infestations in China', paper presented at the American Agricultural Economics Association Meeting, Long Beach, California, USA, 22-26 July. ----- (2008) 'Bt Cotton and Secondary Pests', International Journal of Biotechnology 10(2-3): 113-21

As with other technologies, adoption of Bt seed requires technology specific knowledge. Growing secondary pest populations have slowly eroded the benefits of Bt technology in China. We illustrate the effects of introducing Bt technology among farmers with an imperfect knowledge of secondary pest problems using a simple dynamic model. The stochastic dominance tests based on primary household data from 1999-2001 and 2004 in China provide strong evidence that secondary pests, if unanticipated, could completely erode all benefits from Bt cotton cultivation. Our empirical tests also suggest that planting refuge concurrent with Bt adoption provides for the sustainable development of Bt technology.*

21. Catangui M A and Berg R K (2006): Western bean cutworm, Striacosta albicosta(Smith) (Lepidoptera: Noctuidae), as a potential pest of transgenic Cry1Ab Bacillus thuringiensis corn hybrids in South Dakota. Environmental Entomology 35: 1439-1452.

Injuries caused by the western bean cutworm, Striacosta albicosta (Smith), on transgenic Cry1Ab Bacillus thuringiensis (Bt) corn hybrids were documented and quantiÞed. The western bean cutwormis an emerging or potential pest of transgenic Bt corn in South Dakota. The proportion of ears infested with western bean cutworm larvae in the Cry1Ab Bt corn hybrids were 18Ð20, 38Ð70, and 0Ð34% in 2000, 2003, and 2004, respectively. The Cry1Ab Bt corn hybrids were almost completely free of European corn borer infestations.Untreated conventional corn hybridswere less infestedwith western bean cutworm larvae but more infested with European corn borer larvae. The proportion of ears infestedwithEuropean corn borer larvae alonewere 33, 58Ð80, and 8Ð25%in 2000, 2003, and 2004, respectively. Infestations with western bean cutworm alone were 28, 8Ð28, and 13Ð19%, respectively. Proportion of ears simultaneously infestedwith bothwestern bean cutwormand European corn borer larvae were much lower than single infestations by either species alone, indicating niche overlap and competition. Simultaneous infestations by the two species on untreated conventional corn hybrids were only 8, 0Ð18, and 0Ð1%in 2000, 2003, and 2004. The corn grains harvested frominjured earswere also analyzed for fumonisin and aßatoxin through quantitative enzyme-linked immunosorbent assays. Moremycotoxinswere found in 2003when the levels of insect infestation in the corn earswere higher than in 2004. Results from this study underscore the need to investigate other emerging or potential arthropod pests of transgenic Bt corn hybrids in addition to the western bean cutworm.

22. Kranthi KR, S. Naidu, C.S. Dhawad, A. Tatwawadi, K. Mate, E. Patil, A.A. Bharose, G.T. Behere, R.M. Wadaskar and S. Kranthi (2005): Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, Helicoverpa armigera (Hübner) (Noctuidae: Lepidoptera), Current Science, Vol. 89 (2).

The quantitative levels of Cry1Ac and the seasonal decline in expression differed significantly among the eight commercial Bollgard hybrids tested. The Cry1Ac expression was found to be variable among the hybrids and also between different plant parts. The leaves of Bt-cotton plants were found to have the highest levels of Cry1Ac expression followed by squares, bolls and flowers. The toxin expression in the boll-rind, square bud and ovary of flowers was clearly inadequate to confer full protection to the fruiting parts. Increasing levels of Helicoverpa armigera survival were correlated with the toxin levels decreasing below 1.8 mg/g in the plant parts. Genotype-independent seasonal decline of the Cry1Ac toxin levels was observed in all the hybrids. Cry1Ac expression decreased consistently as the plant aged. The decline in Cry1Ac was more rapid in some hybrids compared to others. The choice of parental background appeared to be crucial for sustainable expression of the cry1Ac transgene. The implications of variability in Cry1Ac expression and the seasonal decline on bollworm management are discussed.

23. Lövei G L & Arpaia S (2005): The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114 (1):114.

We reviewed laboratory tests which studied the impact of genetically modified plants on arthropod natural enemies. A total of 18 species of predators and 14 species of parasitoids have been tested, most in only a few experiments. Certain groups (braconid wasps) or species (the green lacewing, Chrysoperla carnea) have attracted much effort, while representatives of others, including whole orders (e.g., Diptera), have never had a species tested. We conclude that laboratory tests are not the 'worst case' scenarios intended by the experimental designs, and are not often ecologically realistic: they typically provided ad libitum feeding, no prey choice, single prey type, no combination of stress factors and usually uniform temperatures. None of these are representative of field conditions, yet most could be easily mimicked in more complex laboratory tests. In most cases (94.6%), the studies were unable to indicate the level of power required to detect any impact. Small sample size and large variability are factors that mask all but very large differences in potential effects. For predators, 126 parameters were quantified, most commonly including survival/mortality (37 cases), development time (22), and body mass/size (20). For parasitoids, 128 parameters were quantified, the majority involving lectins or proteinase inhibitors. Most frequent measurements were: fecundity (23 experiments), adult longevity, extent of parasitism (17 each), body size, mortality, and larval development time. An aggregative scoring (summarising all quantified parameters) indicated that the laboratory tests quantified a remarkable number of cases (30% for predators, 39.8% for parasitoids), where the impacts of the genetically modified plant were significantly negative. These involve various parameters, organisms, test methods, and significance levels, but collectively they indicate that the use of genetically modified crops may result in negative effects on the natural enemies of crop pests.

24. Men X, Ge F, Edwards C.A. and Yardim E.N. (2005): The influence of pesticide applications on Helicoverpa armigera Hübner and sucking pests in transgenic Bt cotton and non-transgenic cotton in China. Crop Protection 24 (4): 319324.

Effects of pesticide applications, based on an IPM program on cotton bollworm, Helicoverpa armigera Hubner, cotton mirids and cotton leafhoppers, were evaluated in transgenic Bt-cotton and non-transgenic cotton agroecosystems between 1999 and 2001 in China. Differences in pest populations between cotton varieties were also compared. In 1999 and 2000, bollworm populations on non-transgenic cotton were larger than those on transgenic Bt-cotton. In Bt-cotton fields, the numbers of fourth-generation bollworms were greater than those of in the second and the third generations over all 3 years of study. Leafhopper populations on Bt-cotton were consistently larger than those on non-transgenic cotton during the 3 years of study. Although the use of transgenic Bt-cotton decreased the need for insecticide applications against cotton bollworm, this relaxation from pesticide applications could cause increased populations of sucking insects, which could require additional insecticide applications.

25. Chilcutt C H. and Tabashnik B E. (2004): Contamination of refuges by Bacillus thuringensis toxin genes from transgenic maize. Proceedings of the National Academy of Sciences 101:7526-7529.

Transgenic crops producing insecticidal toxins from Bacillus thuringiensis (Bt) are widely used to control pests, but their benefits will be lost if pests evolve resistance. The mandated high-dose/refuge strategy for delaying pest resistance requires planting refuges of toxin-free crops near Bt crops to promote survival of susceptible pests. We report that pollen-mediated gene flow up to 31 m from Bt maize caused low to moderate Bt toxin levels in kernels of non-Bt maize refuge plants. Immunoassays of non-Bt maize sampled from the field showed that the mean concentration of Bt toxin Cry1Ab in kernels and the percentage of kernels with Cry1Ab decreased with distance from Bt maize. The highest Bt toxin concentration in pooled kernels of non-Bt maize plants was 45% of the mean concentration in kernels from adjacent Bt maize plants. Most previous work on gene flow from transgenic crops has emphasized potential effects of transgene movement on wild relatives of crops, landraces, and organic plantings, whereas implications for pest resistance have been largely ignored. Variable Bt toxin production in seeds of refuge plants undermines the high-dose/refuge strategy and could accelerate pest resistance to Bt crops. Thus, guidelines should be revised to reduce gene flow between Bt crops and refuge plants.

26. Tabashnik BE, Gould F and Carriere Y (2004): Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. J Evol Biol. 17(4): 904-12.

The refuge strategy is used widely for delaying evolution of insect resistance to transgenic crops that produce Bacillus thuringiensis (Bt) toxins. Farmers grow refuges of host plants that do not produce Bt toxins to promote survival of susceptible pests. Many modelling studies predict that refuges will delay resistance longest if alleles conferring resistance are rare, most resistant adults mate with susceptible adults, and Bt plants have sufficiently high toxin concentration to kill heterozygous progeny from such matings. In contrast, based on their model of the cotton pest Heliothis virescens, Vacher et al. (Journal of Evolutionary Biology, 16, 2003, 378) concluded that low rather than high toxin doses would delay resistance most effectively. We demonstrate here that their conclusion arises from invalid assumptions about larval concentration-mortality responses and dominance of resistance. Incorporation of bioassay data from H. virescens and another key cotton pest (Pectinophora gossypiella) into a population genetic model shows that toxin concentrations high enough to kill all or nearly all heterozygotes should delay resistance longer than lower concentrations.

27. Huang, F., L. Buschman and R Higgins (1999): Inheritance of resistance to Bacillus thuringiensis toxin (Dipel ES) in the European corn borer. Science 284: 965-966.

Resistance in the European corn borer, Ostrinia nubilalis (Hübner), to a commercial formulation of Bacillus thuringiensis (Bt) Berliner toxin, Dipel ES, appears to be inherited as an incompletely dominant autosomal gene. This contrasts with the inheritance of resistance to Bt in other insects, where it has usually been characterized as a recessive trait. The proposed high-dose/refuge strategy for resistance management in Bt maize depends on resistance being recessive or partially recessive. If field resistance turns out to be similar to this laboratory resistance, the usefulness of the high-dose/refuge strategy for resistance management in Bt maize may be diminished.

28. Gould F and Tabashnik B (1998): Bt cotton Resistance Management. In: Mellon M, Rissler J (eds) Now or Never: Serious new plans to save a natural pest control: 67-106.

29. Hilbeck A, Baumgartner M, Fried PM and Bigler F (1998): Effects of transgenic Bacillus thuringiensis corn-fed prey on mortality and development time of immature Chrysoperla carnea (Neuroptera : Chrysopidae). Environmental Entomology 27 (2): 480-487.

Laboratory feeding experiments were conducted to determine the effects of Bt-fed herbivores (Ostrinia nubilalis and Spodoptera littoralis) on the predator Chrysoperla carnea. Bt corn (HD-1) coding for CryIAb and an isogenic strain were grown in greenhouses and used when they reached 50-80 cm (5-8 leaf stage). Mortality of chrysopid larvae raised on Bt-fed prey was significantly higher (67%) compared to those raised on Bt-free prey (37%). No differences were observed between chrysopid larvae raised on Bt-fed O. nubilalis or S. littoralis. Chrysopid larvae developmental time was prolonged when raised on Bt-fed O. nubilalis but not Bt-fed S. littoralis.

30. Gould F (1998): Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu Rev Entomol. 43: 701-26.

This review examines potential impacts of transgenic cultivars on insect population dynamics and evolution. Experience with classically bred, insecticidal cultivars has demonstrated that a solid understanding of both the target insect's ecology and the cultivar's performance under varied field conditions will be essential for predicting area-wide effects of transgenic cultivars on pest and natural enemy dynamics. This experience has also demonstrated the evolutionary capacity of pests for adaptive response to insecticidal traits in crops. Biochemical and genetic studies of insect adaptation to the Bacillus thuringiensis (Bt) toxins expressed by currently marketed transgenic cultivars indicate a high risk for rapid adaptation if these cultivars are misused. Theoretical and practical issues involved in implementing strategies to delay pest adaptation to insecticidal cultivars are reviewed. Emphasis is placed on examining the "high dose"/refuge strategy that has become the goal of industry and regulatory authorities.

31. Ferro DN (1993): Potential for resistance to Bacillus thuringiensis: Colorado potato beetle (Coleoptera: Chrysomelidae) - a model system. American Entomologist 39:38-44.

A theoretical basis for resistance development to Bacillus thuringiensis by insect pests is presented with examples. The Colorado potato beetle, Leptinotarsa decemlineata (Say), is used as a model organism for discussing potential for resistance development, and operative factors that could influence resistance development are presented. The use of transgenic plants that produce high expression of the delta-endotoxin are likely to select for resistance to this toxin within six generations, based on a simple single-gene model in which a seed mixture of transgenic plants and nontransgenic plants was used. However, if the carrying capacity of the susceptible plant population is included in this model, resistance could take as few as four generations. Plants expressing low levels of toxin, which would inhibit rate of larval development, may provide the best use for transgenic plants; yet, even this approach presents possible problems.

32. McGaughey WH and Whalon ME (1992): Managing insect resistance to Bacillus thuringiensis toxins. Science 258: 1451-1455.

Bacillus thuringiensis (B.t.) delta-endotoxins provide an alternative to chemical insecticides for controlling many species of pest insects. Recent biotechnological developments offer the promise of even greater use of B.t. toxins in genetically transformed pest-resistant crops. However, the discovery that insects can adapt to these toxins raises concerns about the long-term usefulness of B.t. toxins. Several methods for managing the development of resistance to B.t. toxins have been suggested, but none of these approaches offer clear advantages in all situations.

33. Shelton AM Jr, Robertson JL and Tang JD (1993): Resistance of diamondback moth (Lepidoptera: Plutellidae) to Bacillus thuringiensis subspecies in the field. J. Econ. Entomol. 86, 697-705.

Eleven populations of diamondback moth, Plutella xylostella (L.), were collected in 1990 from Brassica plants in six states of the United States and in Indonesia and tested for their responses to two formulations of Bacillus thuringiensis subsp. kurstaki (Javelin WG and DipeI2X), permethrin, and methomyI. Populations from Florida that had been treated extensively over several years with these insecticides displayed significantly higher LC50s. In 1992, field tests in geographically separate areas in Florida and laboratory assays of populations from those fields indicated control failures and resistance to products containing B. thuringiensis subsp. kurstaki and low levels of resistance to a product containing B. thuringiensis subsp. aizawai (XenTari). These B. thuringiensis subspp. differ in the number of toxins produced, but whether resistance to them is a result of cross-resistance or independent selection was not determined. We documented significant differences between the response of resistant and susceptible populations to two products containing B. thuringiensis subsp. kurstaki, thus suggesting that the products actually differed in the number or amounts of toxins. In laboratory bioassays of three products containing B. thuringiensis suhsp. aizawai and two products containing B. thuringiensis subsp. kurstaki, the variation in response (as determined by resistance ratios) varied by 321- to 461-fold for B. thuringiensis subsp. kurstaki and by 3- to 4.1-fold for B. thuringiensis subsp. aizawai. These studies indicate increasing resistance problems caused by intensive use of any B. tlmringiensis product. We conclude that if B. thuringiensis is to remain a durable insecticide in parts of the world where resistance does not already occur, other tactics such as biological control, host-free periods, plant resistance, and cultural controls must he incorporated into the management programs.

AGRI-CHEMICAL USE WITH GM CROPS

1. Charles M Benbrook (2012). Impacts of genetically engineered crops on pesticide use in the U.S. the first sixteen years. Environmental Sciences Europe 2012, 24:24

Genetically engineered, herbicide-resistant and insect-resistant crops have been remarkable commercial successes in the United States. Few independent studies have calculated their impacts on pesticide use per hectare or overall pesticide use, or taken into account the impact of rapidly spreading glyphosate-resistant weeds. A model was developed to quantify by crop and year the impacts of six major transgenic pest-management traits on pesticide use in the U.S. over the 16-year period, 19962011: herbicide-resistant corn, soybeans, and cotton; Bacillus thuringiensis (Bt) corn targeting the European corn borer; Bt corn for corn rootworms; and Bt cotton for Lepidopteron insects. Herbicide-resistant crop technology has led to a 239 million kilogram (527 million pound) increase in herbicide use in the United States between 1996 and 2011, while Bt crops have reduced insecticide applications by 56 million kilograms (123 million pounds). Overall, pesticide use increased by an estimated 183 million kgs (404 million pounds), or about 7%. Contrary to often-repeated claims that today's genetically-engineered crops have, and are reducing pesticide use, the spread of glyphosate-resistant weeds in herbicide-resistant weed management systems has brought about substantial increases in the number and volume of herbicides applied. If new genetically engineered forms of corn and soybeans tolerant of 2,4-D are approved, the volume of 2,4-D sprayed could drive herbicide usage upward by another approximate 50%. The magnitude of increases in herbicide use on herbicide-resistant hectares has dwarfed the reduction in insecticide use on Bt crops over the past 16 years, and will continue to do so for the foreseeable future.

2. Kranthi K R (2010): Bt cotton- A critical appraisal, Annexure to Bt brinjal decision note, Ministry of Environment and Forests, page 180- 190, http://moef.nic.in/downloads/public-information/Annex_BT.pdf along with Kranthi K R (2012): Bt Cotton: Questions & Answers, Indian Society for Cotton Improvement, Mumbai

http://www.downtoearth.org.in/dte/userfiles/images/20l_31072011.jpg

3. Madhura Swaminathan and Vikas Rawal (2011): Are there Benefits from the Cultivation of Bt cotton?. Review of Agrarian Studies Vol 1(1)

This note examines costs and returns from the cultivation of different types of cotton in a rainfed village in the Vidarbha region of Maharashtra, India. While the pros and cons of GM cotton are extensively debated, there are only a few empirical studies on the economic performance of Bt cotton, particularly under rainfed conditions. The results from a detailed survey of farm business incomes show that Bt cotton was a clear leader in terms of production and gross value of output when grown as a stand-alone crop. However, on the fields of small and marginal farmers, where cotton was usually intercropped with sorghum (or other cereals and pulses), the relative income advantage of Bt cotton declined. Further, expenditure on chemical pesticides was higher for Bt cotton than for other varieties of cotton. Variability in production was also higher for Bt cotton than for other types of cotton.

4. Benbrook, C.M. (2009): Impacts of genetically engineered crops on pesticide use in the United States: The first thirteen years. The Organic Center, November.

http://www.organic-center.org/reportfiles/13Years20091126_FullReport.pdf

5. Wang S., Just D. R. and Pinstrup-Andersen P. (2006): 'Tarnishing Silver Bullets: Bt Technology Adoption, Bounded Rationality and the Outbreak of Secondary Pest Infestations in China', paper presented at the American Agricultural Economics Association Meeting, Long Beach, California, USA, 22-26 July. ----- (2008) 'Bt Cotton and Secondary Pests', International Journal of Biotechnology 10(2-3): 113-21

As with other technologies, adoption of Bt seed requires technology specific knowledge. Growing secondary pest populations have slowly eroded the benefits of Bt technology in China. We illustrate the effects of introducing Bt technology among farmers with an imperfect knowledge of secondary pest problems using a simple dynamic model. The stochastic dominance tests based on primary household data from 1999-2001 and 2004 in China provide strong evidence that secondary pests, if unanticipated, could completely erode all benefits from Bt cotton cultivation. Our empirical tests also suggest that planting refuge concurrent with Bt adoption provides for the sustainable development of Bt technology.

6. Arregui MC, Lenardon A, Sanchez D, Maitre MI, Scotta R, Enrique S (2004). Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. Pest Manag Sci. 60:163-166.

The availability of Roundup Ready (RR) varieties of soybean has increased the use of glyphosate for weed control in Argentina. Glyphosate [(N-phosphonomethyl)glycine] is employed for the eradication of previous crop vegetation and for weed control during the soybean growing cycle. Its action is effective, and low environmental impact has been reported so far. No residues have been observed in soil or water, either of glyphosate or its metabolite, AMPA (aminomethylphosphonic acid). The objective of this work was to monitor glyphosate and AMPA residues in soybean plants and grains in field crops in Santa Fe Province, Argentina. Five sites were monitored in 1997, 1998 and 1999. Individual soybean plants were sampled from emergence to harvest, dried and ground. Analysis consisted in residue extraction with organic solvents and buffers, agitation, centrifugation, clean-up and HPLC with UV detection. In soybean leaves and stems, glyphosate residues ranged from 1.9 to 4.4 mg kg(-1) and from 0.1 to 1.8 mg kg(-1) in grains. Higher concentrations were detected when glyphosate was sprayed several times during the crop cycle, and when treatments approached the flowering stage. AMPA residues were also detected in leaves and in grains, indicating metabolism of the herbicide.

7. Pengue, W. (2003): El glifosato y la dominación del ambiente. Biodiversidad 37, July.

http://www.grain.org/biodiversidad/?id=208

8. CASAFE (Camara de Sanidad Agropecuaria y Fertilizantes). Statistics.

http://www.casafe.org.ar/mediciondemercado.html

BIODIVERSITY

1. Bohan DA et al (2005): Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. Proc. R. Soc. B Vol. 272 (1562): 463-474.

We evaluated the effects of the herbicide management associated with genetically modified herbicide-tolerant (GMHT) winter oilseed rape (WOSR) on weed and invertebrate abundance and diversity by testing the null hypothesis that there is no difference between the effects of herbicide management of GMHT WOSR and that of comparable conventional varieties. For total weeds there were few treatment differences between GMHT and conventional cropping, but large and opposite treatment effects were observed for dicots and monocots. In the GMHT treatment, there were fewer dicots and more monocots than in conventional crops. At harvest, dicot biomass and seed rain in the GMHT treatment were one-third of that in the conventional, while monocot biomass was threefold greater and monocot seed rain almost fivefold greater in the GMHT treatment than in the conventional. These differential effects persisted into the following two years of the rotation. Bees and butterflies that forage and select for dicot weeds were less abundant in GMHT WOSR management in July. Year totals for Collembola were greater under GMHT management. There were few other treatment effects on invertebrates, despite the marked effects of herbicide management on the weeds.

2. Hawes, C., Haughton, A.J., Osborne, J.L., Roy, D.B., Clark, S.J., Perry, J.N., Rothery, P., Bohan, D.A., Brooks, D.J., Champion, G.T., Dewar, A.M., Heard, M.S., Woiwod, I.P., Daniels, R.E., Yound, M.W., Parish, A.M., Scott, R.J., Firbank, L.G., Squire, G.R. (2003): Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the farm scale evaluations of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society of London B 358, 18991913.

Effects of genetically modified herbicidetolerant (GMHT) and conventional crop management on invertebrate trophic groups (herbivores, detritivores, pollinators, predators and parasitoids) were compared in beet, maize and spring oilseed rape sites throughout the UK. These trophic groups were influenced by season, crop species and GMHT management. Many groups increased twofold to fivefold in abundance between early and late summer, and differed up to 10fold between crop species. GMHT management superimposed relatively small (less than twofold), but consistent, shifts in plant and insect abundance, the extent and direction of these effects being dependent on the relative efficacies of comparable conventional herbicide regimes. In general, the biomass of weeds was reduced under GMHT management in beet and spring oilseed rape and increased in maize compared with conventional treatments. This change in resource availability had knockon effects on higher trophic levels except in spring oilseed rape where herbivore resource was greatest. Herbivores, pollinators and natural enemies changed in abundance in the same directions as their resources, and detritivores increased in abundance under GMHT management across all crops. The result of the later herbicide application in GMHT treatments was a shift in resource from the herbivore food web to the detritivore food web. The Farm Scale Evaluations have demonstrated over 3 years and throughout the UK that herbivores, detritivores and many of their predators and parasitoids in arable systems are sensitive to the changes in weed communities that result from the introduction of new herbicide regimes.

3. Brooks, D.R., Bohan, D.A., Champion, G.T., Haughton, A.J., Hawes, C., Heard, M.S., Clark, S.J., Dewar, A.M., Firbank, L.G., Perry, J.N., Rothery, P., Scott, R.J., Woiwod, I.P., Birchall, C., Skellern, M.P., Walker, J.H., Baker, P., Bell, D., Browne, E.L., Dewar, A.J.D., Fairfax, C.M., Garner, B.H., Haylock, L.A., Horne, S.L., Hulmes, S.E., Mason, N.S., Norton, L.P., Nuttall, P., Randall, Z., Rossall, M.J., Sands, R.J.N., Singer, E.J., Walker, M.J. (2003): Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface active invertebrates. Philosophical Transactions of the Royal Society of London B 358, 18471862.

The effects of herbicide management of genetically modified herbicidetolerant (GMHT) beet, maize and spring oilseed rape on the abundance and diversity of soilsurfaceactive invertebrates were assessed. Most effects did not differ between years, environmental zones or initial seedbanks or between sugar and fodder beet. This suggests that the results may be treated as generally applicable to agricultural situations throughout the UK for these crops. The direction of the effects was evenly balanced between increases and decreases in counts in the GMHT compared with the conventional treatment. Most effects involving a greater capture in the GMHT treatments occurred in maize, whereas most effects involving a smaller capture were in beet and spring oilseed rape. Differences between GMHT and conventional crop herbicide management had a significant effect on the capture of most surfaceactive invertebrate species and higher taxa tested in at least one crop, and these differences reflected the phenology and ecology of the invertebrates. Counts of carabids that feed on weed seeds were smaller in GMHT beet and spring oilseed rape but larger in GMHT maize. In contrast, collembolan detritivore counts were significantly larger under GMHT crop management.

4. Hilbeck A (2001): Implications of transgenic, insecticidal plants for insect and plant biodiversity. Perspectives in Plant Ecology, Evolution and Systematics, 4(1), 43-61

In this paper possible implications of non-target effects for insect and plant biodiversity are discussed and a case example of such non-target effects is presented. In a multiple year research project, tritrophic and bitrophic effects of transgenic corn, expressing the gene from Bacillus thuringiensis (Bt-corn) that codes for the high expression of an insecticidal toxin (Cry1Ab), on the natural enemy species, Chrysoperla carnea (the green lacewing), was investigated. In these laboratory trials, we found prey-mediated effects of transgenic Bt-corn causing significantly higher mortality of C. carnea larvae. In further laboratory trials, we confirmed that the route of exposure (fed directly or via a herbivorous prey) and the origin of the Bt (from transgenic plants or incorporated into artificial diet) strongly influenced the degree of mortality. In choice feeding trials where C. carnea could choose between Spodoptera littoralis fed transgenic Bt-corn and S. littoralis fed non-transgenic corn, larger instars showed a significant preference for S. littoralis fed non-transgenic corn while this was not the case when the choice was between Bt- and isogenic corn fed aphids. Field implications of these findings could be multifold but will be difficult to assess because they interfere in very intricate ways with complex ecosystem processes that we still know only very little about. The future challenge in pest management will be to explore how transgenic plants can be incorporated as safe and effective components of IPM systems and what gene technology can contribute to the needs of a modern sustainable agriculture that avoids or reduces adverse impacts on biodiversity? For mainly economically motivated resistance management purposes, constitutive high expression of Bt-toxins in transgenic plants is promoted seeking to kill almost 100% of all susceptible (and if possible heterozygote resistant) target pest insects. However, for pest management this is usually not necessary. Control at or below an established economic injury level is sufficient for most pests and cropping systems. It is proposed that partially or moderately resistant plants expressing quantitative rather than single gene traits and affecting the target pest sub-lethally may provide a more meaningful contribution of agricultural biotechnology to modern sustainable agriculture. Some examples of such plants produced through conventional breeding are presented. Non-target effects may be less severe allowing for better incorporation of these plants into IPM or biological control programs using multiple control strategies, thereby, also reducing selection pressure for pest resistance development.

5. Watkinson AR, Freckleton RP, Robinson RA & W J Sutherland (2000): Predictions of biodiversity response to genetically modified herbicide-tolerant crops. Science 289:1554-1557

It has been suggested that genetically modified herbicidetolerant crops may benefit biodiversity because spraying of crops may be delayed until later in the growing season, allowing weeds to grow during the early part of the year. This provides an enhanced resource for arthropods, and potentially benefits birds that feed on these. Thus, this technology could enhance biodiversity. Using a review of weed phenologies and a population model, we show that many weeds are unlikely to benefit because spraying is generally delayed insufficiently late in the season to allow most to set seed. The positive effects on biodiversity observed in trials lasting one or two seasons are thus likely to be transient. For one weed of particular significance (Chenopodium album, fat hen) we show that it is unlikely that the positive effects observed could be maintained by inputs of seed during other parts of the rotation. However, we find preliminary evidence that if spraying can be ceased earlier in the season, then a viable population of lateemerging weeds could be maintained. This strategy could benefit weeds in both genetically modified (GM) and nonGM crops, but would probably lead to reduced inputs in GM systems compared with conventional ones.

SOIL IMPACTS

1. Jagadish C. Tarafdar, Indira Rathore and Vandana Shiva (2012): Effect of Bt- transgenic cotton on soil biological health. Applied Biological Research 14 (1): 15-23

Bt cotton are plants that have been genetically modified to express the insecticidal proteins Cry 1 Ac from subspecies of the bacterium, Bacillus thuringiensis israelensis (Bt), to control bollworm pest that feed on cotton. There is a persistent environmental concern that transgenic Bt-crops carry genes that have indirect undesirable effect to natural and agroecosystem function. We investigated the effect of Bt-cotton (with Cry 1 Ac gene) on several microbial and biochemical indicators in fields under sub-humid tropical condition. Twenty five fields were selected in the Vidarbha region, India, where Bt-cotton has been growing at least three consecutive years and side by side field of non-transgenic cotton is growing under clay to clay loam soil. Soil from a control (no-crop) treatment was also included from each area to compare the extent of adverse effect of Bt, if any. Samples were analyzed for actinobacteria, fungi and nitrifiers population, biomass carbon (MBC), biomass nitrogen (MBN), biomass phosphorus (MBP) and soil enzyme activities. The result revealed a significant decline in actinobacteria (17%), bacterial (14%) count as well as acid phosphatase (27%), phytase (18%), nitrogenase (23%) and dehydrogenase (12%) activities in Bt cotton compared with non-Bt cotton fields. Fungal and nitrifier counts, and esterase and alkaline phosphatase activities were not affected by the introduction of Bt-cotton in fields. However, significant decline between 8 and 9% in MBC and MBN was noticed.

2. Cheeke TE, Rosenstiel TN, Cruzan MB. (2012): Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of Bt maize. American Journal of Botany. 99(4): 700707.

Insect-resistant Bacillus thuringiensis (Bt) maize is widely cultivated, yet few studies have examined the interaction of symbiotic arbuscular mycorrhizal fungi (AMF) with different lines of Bt maize. As obligate symbionts, AMF may be sensitive to genetic changes within a plant host. Previous evaluations of the impact of Bt crops on AMF have been inconsistent, and because most studies were conducted under disparate experimental conditions, the results are difficult to compare. We evaluate AMF colonization in nine Bt maize lines, differing in number and type of engineered trait, and five corresponding near-isogenic parental (P) base hybrids in greenhouse microcosms. Plants were grown in 50% local agricultural soil with low levels of fertilization, and AMF colonization was evaluated at 60 and 100 d. Nontarget effects of Bt cultivation on AMF colonization were tested in a subsequently planted crop, Glycine max, which was seeded into soil that had been preconditioned for 60 d with Bt or P maize. We found that Bt maize had lower levels of AMF colonization in their roots than did the non-Bt parental lines. However, reductions in AMF colonization were not related to the expression of a particular Bt protein. There was no difference in AMF colonization in G. max grown in the Bt- or P-preconditioned soil. These findings are the first demonstration of a reduction in AMF colonization in multiple Bt maize lines grown under the same experimental conditions and contribute to the growing body of knowledge examining the unanticipated effects of Bt crop cultivation on nontarget soil organisms.

3. Chen ZH, Chen LJ, Zhang YL, WuZJ (2011): Microbial properties, enzyme activities and the persistence of exogenous proteins in soil under consecutive cultivation of transgenic cottons (Gossypium hirsutum L.), Plant Soil and Environment UZEI 57 (2): 67-74

One Bacillus thuringiensis (Bt) and two stacked Bt and cowpea trypsin inhibitor (Bt + CpTI) cottons and their non-transgenic isolines were consecutively cultivated to investigate the soil persistence of Cry1Ac and CpTI proteins and their effects on microbial properties and enzyme activities involving C, N, P, and S cycling in soil. Cry1Ac and CpTI proteins persisted in soil under transgenic cottons cultivated consecutively during 4 years. The concentration of Cry1Ac proteins varied from 6.75 ng/g to 12.01 ng/g and that of CpTI proteins from 30.65 to 43.60 ng/g. However, neither of these two proteins was detected in soil under non-transgenic cottons. Soil microbial biomass carbon, microbial activities, and soil enzyme activities (except urease and phosphodiesterase) significantly decreased in soil under transgenic cottons. Correlation analysis showed that most of microbial properties and enzyme activities in soil had a negative relationship with Cry1Ac content, while most of them had a positive relationship with CpTI content. Our data indicate that consecutive cultivation by genetically modified cottons with Bt and CpTI genes can result in persistence of Cry1Ac and CpTI proteins and negatively affect soil microbial and biochemical properties.

4. Chen Z H, Chen L J, Zhang Y L, Wu Z J. (2011): Microbial properties, enzyme activities and persistence of exogenous proteins in soil under consecutive cultivation of transgenic cottons (Gossypium hirsutum L.), Plant Soil Environ. 57 (2): 67-74

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www.agriculturejournals.cz/publicFiles/35214.pdf

5. Yuan YG, Ge F. (2010): Effects of transgenic Bt crops on non-target soil animals. [Article in Chinese]; Ying Yong Sheng Tai Xue Bao. (Journal of Applied Ecology) 21(5):1339-45.

Transgenic Bt crops are widely planted around the world. With the quick development and extension of genetically modified crops, it is needed to make a deep study on the effects of Bt crops on soil ecosystem. This paper reviewed the research progress on the effects of transgenic Bt crops on the population dynamics and community structure of soil animals, e.g., earthworm, nematode, springtail, mite, and beetle, etc. The development history of Bt crops was introduced, the passway the Bt protein comes into soil as well as the residual and degradation of Bt protein in soil were analyzed, and the critical research fields about the ecological risk analysis of transgenic Bt crops on non-target soil animals in the future were approached, which would provide a reference for the research of the effects of transgenic Bt crops on non-target soil animals.

6. Kremer R.J and Means N.E. (2009): Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. European Journal of Agronomy 31 (3): 153161.

Current crop production relies heavily on transgenic, glyphosate-resistant (GR) cultivars. Widespread cultivation of transgenic crops has received considerable attention. Impacts of glyphosate on rhizosphere microorganisms and activities are reviewed based on published and new data from long-term field projects documenting effects of glyphosate applied to GR soybean and maize. Field studies conducted in Missouri, U.S.A. during 19972007 assessed effects of glyphosate applied to GR soybean and maize on root colonization and soil populations of Fusarium and selected rhizosphere bacteria. Frequency of root-colonizing Fusarium increased significantly after glyphosate application during growing seasons in each year at all sites. Roots of GR soybean and maize treated with glyphosate were heavily colonized by Fusariumcompared to non-GR or GR cultivars not treated with glyphosate. Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen antagonistic bacterial interactions; and reduction in nodulation. Root-exuded glyphosate may serve as a nutrient source for fungi and stimulate propagule germination. The specific microbial indicator groups and processes were sensitive to impacts of GR crops and are part of an evolving framework in developing polyphasic microbial analyses for complete assessment of GR technology that is more reliable than single techniques or general microbial assays.

http://naldc.nal.usda.gov/download/35795/PDF

7. Johal G.S and Huber D.M. (2009): Glyphosate effects on diseases of plants. Europ. J. Agronomy 31, 144152.

Glyphosate, N-(phosphonomethyl)glycine, is the most extensively used herbicide in the history of agriculture. Weed management programs in glyphosate resistant (GR) field crops have provided highly effective weed control, simplified management decisions, and given cleaner harvested products. However, this relatively simple, broad-spectrum, systemic herbicide can have extensive unintended effects on nutrient efficiency and disease severity, thereby threatening its agricultural sustainability. A significant increase. in disease severity associated with the wide spread application of the glyphosate herbicide can be the result of direct glyphosate-induced weakening of plant defenses and increased pathogen population and virulence. Indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients involved in physiological disease resistance. Strategies to ameliorate the predisposing effects of glyphosate on disease include judicious selection of herbicide application rates, micronutrient amendment, glyphosate detoxification in meristematic tissues and soil, changes in cultural practices to enhance micronutrient availability for plant uptake, and biological amendment with glyphosate-resistant microbes for nitrogen fixation and nutrient availability. Given that recommended doses of glyphosate are often many times higher than needed to control weeds, we believe the most prudent method to reduce the detrimental effects of glyphosate on GR crops will be to use this herbicide in as small a dose as practically needed. Such a frugal approach will not only curtail disease predisposition of GR crops, but will also benefit the grower and the environment.

http://www.certifiedorganic.bc.ca/rcbtoa/services/huber-glyphosates-2009.pdf

8. Fernandez M.R, Zentner R.P, Basnyat P, Gehl D, Selles F and Huber D (2009): Glyphosate associations with cereal diseases caused by Fusarium spp. in the Canadian prairies. Eur. J. Agron. 31, 133143.

Fusarium pathogens cause important diseases, such as root/crown rot and Fusarium head blight (FHB), in cereal crops. These diseases can be caused by similar Fusarium spp. Common root rot (CRR) is widespread in the western Canadian Prairies, whereas FHB has potential of becoming an important disease in this region. There are no commercially available cereal cultivars with good resistance to these diseases. It is therefore important to identify agronomic practices that could affect levels of Fusarium pathogens in cereals. This review deals primarily with the effects of tillage systems and glyphosate use on the development of FHB and CRR in wheat and barley in eastern Saskatchewan. Although the FHB study in 1999-2002 indicated that environment was the most important factor determining FHB development, previous glyphosate use and tillage practice were among the production factors with the greatest association with FHB. Overall, disease was highest in crops under minimum-till management. Previous glyphosate use was consistently associated with higher FHB levels caused by the most important FHB pathogens, Fusarium avenaceum and Fusarium graminearum. Cochliobolus sativus, the most common CRR pathogen, was negatively associated with previous glyphosate use, while F. avenaceum, F. graminearum, and other fungi were positively associated, suggesting that glyphosate might cause changes in fungal communities. The occurrence and isolation of F. avenaceum from cereal residues were greater under reduced-till than conventional-till while C. sativus was most common under conventional-till, and F. graminearum was lowest under zero-till. Previous glyphosate applications were again correlated positively with F. avenaceum and negatively with C. sativus. These observations agreed with results from the FHB and CRR studies. These are the first studies that established a relationship between previous glyphosate use and increased Fusarium infection of spikes and subcrown internodes of wheat and barley, or Fusarium colonization of crop residues. However, because of the close association between noncereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on Fusarium infections. Determining the relative contribution of these popular production trends to the development of diseases caused by Fusarium spp. are essential for devising appropriate agronomic recommendations to prevent their further spread in western Canada, and to reduce the impact that these diseases are having in areas where they are already established. The consistent association between previous glyphosate use and Fusarium infections also warrants further research to elucidate the nature of this association and the underlying mechanisms determining these effects.

http://www.ars.usda.gov/sp2UserFiles/Place/36221500/cswq-0426-yamada.pdf

9. Liu W (2009): Effects of Bt transgenic crops on soil ecosystems: a review of a 10- year research in China. Front. Agric. China 3(2): 190-98

Bacillus thuringiensis (Bt) transgenic cotton is the unique Bt transgenic crop planted on a large scale in China, and its commercialized varieties and hectareage had increased rapidly in China during the past decade (19972006) with broad geographic distribution for the economic, environmental, and health benefits. In 2004, the planting area of Bt transgenic cotton in China ranked first worldwide with up to 370 × 106 hm2. In addition, Bt transgenic rice varieties in field tests have been close to approval for commercialization. However, ecological risks, a complex issue of Bt transgenic crops on soil ecosystem is urgently faced in China due to more than 60 varieties transferred single or bivalent Bt genes grown under diverse geographic regions. Two main pathways, biomass incorporation and root exudates, are involved in the effects of Bt transgenic crops on soil ecosystems. In this paper, the research results in recent years in China involved in the effects of Bt transgenic crops (Bt transgenic cottons and rice) on soil ecosystems were summarized with special attentions paid to the release and persistence of Bt toxins, and the toxicology to microorganisms, as well as the change of soil biochemical properties in soils where Bt transgenic crops were planted or incubated with their biomass. In addition, the complexity and current research defaults of ecological risk evaluation of Bt transgenic crops in China were highlighted.

http://rd.springer.com/article/10.1007/s11703-009-0027-9

10. Höss S, Arndt M, Baumgarte S, Tebbe C.C, Nguyen H.T. and Jehle J.A. (2008): Effects of transgenic corn and Cry1Ab protein on the nematode, Caenorhabditis elegans. Ecotoxicology and Environmental Safety 70 (2): 334340.

The effects of the insecticidal Cry1Ab protein from Bacillus thuringiensis (Bt) on the nematode, Caenorhabditis elegans, were studied with soil from experimental fields cultivated with transgenic Bt corn (MON810) and with trypsinized Cry1Ab protein expressed in Escherichia coli. The content of Cry1Ab protein was above the detection limit of an ELISA test in only half of the soil samples obtained from transgenic plots, ranging from 0.19 to 1.31 ng g 1dry weight. In a laboratory bioassay, C. elegans was exposed to rhizosphere and bulk soil from fields with isogenic or transgenic corn or to solutions of Cry1Ab protein (0, 24, 41, 63, 118, and 200 mg l1) over a period of 96 h, with growth and reproduction serving as the test parameters. Nematode reproduction and growth were significantly reduced in rhizosphere and bulk soil of Bt corn compared with soil from isogenic corn and were significantly correlated with concentrations of the Cry1Ab protein in the soil samples. Moreover, the toxicity of pure Cry1Ab protein to the reproduction and growth of C. elegans was concentration-dependent. As significant inhibition occurred at relatively high concentrations of the Cry1Ab protein (41 mg l1), the effects of the soil samples from Bt corn could not be assigned directly to the toxicity of the Cry1Ab protein. The results demonstrate that bioassays with the nematode, C. elegans, provide a promising tool for monitoring the potential effects of Bt toxins in aqueous medium and soils.

11. Icoz I. & Stotzky G. (2008): Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40: 559586.

Recent applications of biotechnology, especially genetic engineering, have revolutionized crop improvement and increased theavailability of valuable new traits. A current example is the use of the insecticidal Cry proteins from the bacterium, Bacillus thuringiensis (Bt), to improve crops, known as Bt crops, by reducing injury from various crop pests. The adoption of genetically modified (GM) cropshas increased dramatically in the last 11 years. However, the introduction of GM plants into agricultural ecosystems has raised a numberof questions, including the ecological impact of these plants on soil ecosystems. Crop residues are the primary source of carbon in soil, and root exudates govern which organisms reside in the rhizosphere. Therefore, any change to the quality of crop residues and rhizosphere inputs could modify the dynamics of the composition and activity of organisms in soil. Insect-resistant Bt crops have the potential to change the microbial dynamics, biodiversity, and essential ecosystem functions in soil, because they usually produce insecticidal Cry proteins through all parts of the plant. It is crucial that risk assessment studies on the commercial use of Bt crops consider the impacts on organisms in soil. In general, few or no toxic effects of Cry proteins on woodlice, collembolans, mites, earthworms, nematodes, protozoa, and the activity of various enzymes in soil have been reported. Although some effects, ranging from no effect to minor and significant effects, of Bt plants on microbial communities in soil have been reported, using both culturing and molecular techniques, they were mostly the result of differences in geography, temperature, plant variety, and soil type and, in general, were transient and not related to the presence of the Cry proteins. The respiration (i.e., CO2 evolution) of soils cultivated with Bt maize or amended with biomass of Bt maize and other Bt crops was generally lower than from soils cultivated with or amended with biomass of the respective non-Bt isolines, which may have been a result of differences in chemical composition (e.g., the content of starch, soluble N,proteins, carbohydrates, lignin) between Bt

plants and their near-isogenic counterparts. Laboratory and field studies have shown differences in the persistence of the Cry proteins in soil, which appear to be the result primarily of differences in microbial activity, which, in turn, is dependent on soil type (e.g., pH, clay mineral composition, other physicochemical characteristics), season (e.g., temperature, water tension), crop species (e.g., chemical composition, C:N ratio, plant part), crop management practices (e.g., till vs. no-till), and other environmental factors that vary with location and climate zones. This review discusses the available data on the effects of Cry proteins on below-ground organisms, the fate of these proteins in soil, the techniques and indicators that are available to study these aspects, and future directions

http://www.cof.orst.edu/cof/teach/agbio2011/Other%20Readings/Icoz_SoilBio_2008_Fate%20effects%20of%20Bt%20crops%20in%20soil.pdf

12. Sarkar B, Patra AK and Purakayastha TJ (2008): Transgenic Bt-Cotton Affects Enzyme Activity and Nutrient Availability in a Sub-Tropical Inceptisol. J. Agronomy & Crop Science (2008) ISSN 0931-2250

We investigated the dynamics of N and P availability in the rhizosphere of Bt and non-Bt cotton crops during their growth. In a net-house pot culture experiment at the Indian Agricultural Research Institute, New Delhi, Bt-cotton (cv. MRC-6301Bt) and its non-transgenic near-isoline (MRC-6301) were grown on a sandy loam soil until maturity. A control (no-crop) treatment was also included. Rhizosphere soil and root samples were collected at 60, 90, and 120 days after sowing (DAS). Soil samples were analysed for dehydrogenase activity, soil respiration, mineral-N and Olsen-P. Results have revealed a significant reduction in dehydrogenase activity (−17 %) and soil respiration (−3.5 %) in the rhizosphere of Bt-cotton over non-Bt isoline. Total mineral-N (NH4+-N + -N) in soil was reduced by 14 %, whereas Olsen-P was increased by 8 % because of Bt-cotton. Root biomass yields were not different ($P > 0.05$), but root volume was significantly higher in Bt than non-Bt isoline. Time of sampling strongly ($P < 0.05$) affected the above parameters, showing their highest values at 60 or 90 DAS. A significant interactive effect of sampling time and treatments was also indicated. Our results suggest that Bt-cotton may constrain the availability of N, but enhances P-availability in these soils.

13. Mulder C, Wouterse M, Rutgers M. & Posthuma L. (2007): Transgenic maize containing the Cry1Ab protein ephemerally enhances soil microbial communities Ambio 36: 359-361.

Besides reports of facultative phytophagous insects that seem to have acquired resistance to larvicidal toxins belonging to the Cry1 protein family, nontarget effects of the toxin produced by the insecticidal cry1Ab gene released in the root exudates of B. thuringiensis maize (Zea mays L.) are unclear. Microbial communities occurring below ground under genetically engineered Bt-crops are less investigated, and possible effects on microbes remain a concern. Toxins may accumulate in soil after postharvest maize straw is plowed under (a common practice in agriculture), and high concentrations of the Cry1Ab toxin seem to persist for several months (5). In this paper, the following questions will be addressed: Is there microbial evidence of environmental disturbance in relation to plowed leaves and straw of Bt-maize in soils? Can a better ecological insight in the microbial community be obtained using metabolic fingerprints of soil bacteria?

http://sd-cite.iisd.org/cgi-bin/koha/opac-detail.pl?biblionumber=39026

14. Bruns H.A. & Abel C.A. (2007): Effects of nitrogen fertility on Bt endotoxin levels in corn. Journal of Entomological Science 42 (1): 3534

Some corn hybrids have been modified through biotechnology and have a gene inserted into them from bacteria called Bacillus thuringiensis (Bt). This causes the plant to produce a natural toxin that kills the caterpillars of southwestern corn borer and other similar insect pests. The U.S. Environmental Protection Agency recommends that corn with a Bt gene be grown with sufficient nitrogen fertilizer to produce high levels of Bt-toxin and prevent the insects from surviving and developing resistance to it. When corn hybrids with a MON-810 Bt gene had five fully extended leaves, Bt-toxin levels in the plants increased as the nitrogen fertilizer rates increased from 0 to 100, 200, and 300 pounds per acre. A corn hybrid with a DBT-418 Bt gene had no such increases in Bt-toxin at this early growth stage, but its plant tissue was as fatal as tissue from MON-810 Bt hybrids when fed to southwestern corn borer caterpillars. Later when the developing corn kernels were at the milk stage of growth, MON 810 hybrids still had consistent increases in the Bt-toxin in ear husks and ear-leaf sheaths as nitrogen fertilizer rates increased. Southwestern corn borer caterpillars all died when fed plant tissue collected at all nitrogen fertility rates. The DBT 418 Bt hybrid had similar increases in Bt-toxin in the same plant tissues as nitrogen fertilizer rates increased. However, southwestern corn borer caterpillars were able to survive on these plant tissues. Despite the differences in sensitivity of southwestern corn borer caterpillars to these two Bt-toxins during kernel development, this experiment demonstrates that, during this growth period, corn containing a Bt gene will have higher levels of Bt-toxin as nitrogen fertilizer rates are increased.

15. Fernandez M.R, Zentner R.P, DePauw R.M, Gehl D, Stevenson F.C. (2007): Impacts of crop production factors on common root rot of barley in Eastern Saskatchewan. Crop Sci. 47, 15851595.

Fusarium head blight (FHB) in barley (Hordeum vulgare L.) has been spreading on the Canadian Prairies for the last decade. Fusarium spp. causing FHB can also cause crown and root rot of cereal crops. It is therefore of interest to determine the impact of agronomic practices on fungal populations associated with root rot of barley. From 1999 to 2001, 137 barley crops were sampled in eastern Saskatchewan for severity of subcrown internode discoloration and percentage isolation of fungi. Cochliobolus sativus was the most commonly isolated fungus, whereas the most commonly isolated Fusarium spp. included the FHB pathogens F. avenaceum, F. culmorum, and F. graminearum Discoloration caused by C. sativus was favored by conventional-till, whereas Fusarium spp. increased in reduced tillage systems. Barley grown after a cerealsummer fallow sequence under conventional- or minimum-till had increased levels of C. sativus. Fusarium spp. were most affected by the previously grown crop(s); they were more common in barley grown after a noncereal than a cereal, and after two noncereals, or a noncereal alternated with summer fallow. Previous glyphosate applications were associated with lower C. sativus and higher Fusarium spp. levels in barley grown under minimum-till management. This suggests changes in fungal communities; however, the mechanism(s) responsible for these changes in fungal levels are not known. Increased infection of ground and underground tissue by FHB pathogens may contribute to its development in succeeding cereal crops. Therefore, measures aimed at reducing root and crown infections by Fusarium spp. may also help reduce FHB development.

https://www.crops.org/publications/cs/Abstracts/47/4/1585

16. Neumann G, Kohls S, Landsberg E, Stock-Oliveira Souza K, Yamada T, Romheld V (2006): Relevance of glyphosate transfer to non-target plants via the rhizosphere. Journal of Plant Diseases and Protection 20: 963-969.

There is a common understanding that the widely used herbicide glyphosate is easily degraded and adsorbed in soils and thus, harmless for use in agriculture. We can demonstrate, however, that this conclusion is wrong and dangerous for farmers because in former risk assessments the behaviour of glyphosate in the rhizosphere was not properly considered. In nutrient solution, rhizobox and pot experiments we can show that foliar applied glyphosate to target plants is released into the rhizosphere after a fast translocation from shoots to roots. In the rhizosphere glyphosate can obviously be stabilized long enough to achieve negative effects on non-target plants. Such a negative side effect is for example inhibited acquisition of micronutrients such as Mn, but also Zn, Fe and B, which are involved in plant own disease resistance mechanisms. From this glyphosate transfer from target to non-target plants (e.g. from weed to trees in orchards) we predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the USA. In view of plant and soil health, we urgently call for a re-assessment of glyphosate as herbicide.

http://www.jpdp-online.com/Artikel.dll/02-Roemheld_MTAyNzEw.PDF

17. Sun, X, L. J. Chen, Z. J. Wu, L. K. Zhou and H. Shimizu (2006): Soil persistence of Bacillus thuringiensis (Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzyme activities. Biology and Fertility of Soils 43 (5): 617-620.

A silty loam soil was incubated with the leaves and stems of two transgenics Bacillus thuringiensis (Bt) cotton varieties and nontransgenic Bt cotton to study the soil persistence of the Bt toxin from the decomposing transgenic Bt cotton tissues and its effect on soil enzyme activities. The results showed that after bt cotton tissue amendment, Bt toxin was introduced into soil upon decomposition; about 50% of the introduced Bt toxin persisted in soil for at least 56 days. No Bt toxin was detected in the nontransgenic Bt cotton-amended soil; the amount of Bt toxin was the highest in the soil treated with the residue with the higher Bt toxin content. Activities of soil urease, acid phosphomonoesterase, invertase, and cellulase were stimulated by the addition of Bt cotton tissues, whereas activity of soil arylsulfatase was inhibited. Probably cotton tissue stimulated microbial activity in soil, and as a consequence, enzyme activities of soil were generally increased. This effect can mask any negative effect of the bt toxin on microbial activity and thus on enzyme activities.

http://journals.ohiolink.edu/ejc/search.cgi?q=authorExact:%22Sun%2C%20C.%20X.%22

18. Gordon B (2006): Manganese nutrition of glyphosate resistant and conventional soybeans. Better Crops 91 (4): 12-13.

This study was conducted to determine if glyphosate-resistant (GR) soybeans respond differently to Mn fertilizer than conventional soybean varieties in an irrigated high-yield environment, and if so to develop fertilization strategies that will prevent or correct deficiencies. Yield of the GR variety was less than the conventional variety without Mn fertilizer.However, Mn application (banded at planting) to the GR variety closed the yield gap. The conventional soybean variety was not responsive to Mn fertilization. Conversely, yield was reduced at the highest rate of Mn. A second phase of the study showed that a combination of Mn applied as starter and foliar application provided maximum yield response.

19. Wei XD, Zou HL, Chu LM, Liao B, Ye CM and Lan CY (2006): Field-released transgenic papaya affects microbial communities and enzyme activities in soil. J. Environ Sci (China) 18 (4): 734-40 as well as in Plant and Soil Volume 285, Numbers 1-2, 347-358.

Soil properties, microbial communities and enzyme activities were studied in soil amended with replicase (RP)-transgenic or non-transgenic papaya under field conditions. Compared with non-transgenic papaya, significant differences ($P<0.05$) were observed in total nitrogen in soils grown with transgenic papaya. There were also significant differences ($P<0.05$) in the total number of colony forming units (CFUs) of bacteria, actinomycetes and fungi between soils amended with RP-transgenic plants and non-transgenic plants. Compared with non-transgenic papaya, the total CFUs of bacteria, actinomycetes and fungi in soil with transgenic papaya increased by 0.43-1.1, 0.21-0.80 and 0.46-0.73 times respectively. Significantly higher ($P<0.05$) CFUs of bacteria, actinomycetes and fungi resistant to kanamycin (Km) were obtained in soils with RP-transgenic papaya than those with non-transgenic papaya in all concentrations of Km. Higher resistance quotients for Kmr (kanamycin resistant) bacteria, actinomycetes and fungi were found in soil planted with RP-transgenic papaya, and the resistance quotients for Kmr bacteria, actinomycetes and fungi in soils with transgenic papaya increased 1.6-4.46, 0.63-2.5 and 0.75-2.30 times. RP-transgenic papaya and non-transgenic papaya produced significantly different enzyme activities in arylsulfatase (5.4-5.9x), polyphenol oxidase (0.7-1.4x), invertase (0.5-0.79x), cellulase (0.23-0.35x) and phosphodiesterase (0.16-0.2x). The former three soil enzymes appeared to be more sensitive to the transgenic papaya than the others, and could be useful parameters in assessing the effects of transgenic papaya. Transgenic papaya could alter soil chemical properties, enzyme activities and microbial communities.

http://www.ncbi.nlm.nih.gov/pubmed/17078553

20. Mulder C, Wouterse M, Raubuch M, Roelofs W & Rutgers M. (2006): Can transgenic maize affect soil microbial communities? PLoS Computational Biology 2 (9): 11651172.

The aim of the experiment was to determine if temporal variations of belowground activity reflect the influence of the Cry1Ab protein from transgenic maize on soil bacteria and, hence, on a regulatory change of the microbial community (ability to metabolize sources belonging to different chemical guilds) and/or a change in numerical abundance of their cells. Litter placement is known for its strong influence on the soil decomposer communities. The effects of the addition of crop residues on respiration and catabolic activities of the bacterial community were examined in microcosm experiments. Four cultivars of Zea mays L. of two different isolines (each one including the conventional crop and its Bacillus thuringiensis cultivar) and one control of bulk soil were included in the experimental design. The growth models suggest a dichotomy between soils amended with either conventional or transgenic maize residues. The Cry1Ab protein appeared to influence the composition of the microbial community. The highly enhanced soil respiration observed during the first 72 h after the addition of Bt-maize residues can be interpreted as being related to the presence of the transgenic crop residues. This result was confirmed by agar plate counting, as the averages of the colony-forming units of soils in conventional treatments were about one-third of those treated with transgenic straw. Furthermore, the addition of Bt-maize appeared to induce increased microbial consumption of carbohydrates in BIOLOG EcoPlates. Three weeks after the addition of maize residues to the soils, no differences between the consumption rate of specific chemical guilds by bacteria in soils amended with transgenic maize and bacteria in soils amended with conventional maize were detectable. Reaped crop residues, comparable to post-harvest maize straw (a common practice in current agriculture), rapidly influence the soil bacterial cells at a functional level. Overall, these data support the existence of short Bt-induced ecological shifts in the microbial communities of croplands' soils.

http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.

21. Castaldini M, Turrini A, Sbrana C, Benedetti A, Marchionni M, Mocali S, Fabiani A, Landi S, Santomassimo F, Pietrangeli B, Nuti MP, Miclaus N and Giovannetti M (2005): Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Appl Environ Microbiol. 71(11): 6719-29.

A polyphasic approach has been developed to gain knowledge of suitable key indicators for the evaluation of environmental impact of genetically modified Bt 11 and Bt 176 corn lines on soil ecosystems. We assessed the effects of Bt corn (which constitutively expresses the insecticidal toxin from Bacillus thuringiensis, encoded by the truncated Cry1Ab gene) and non-Bt corn plants and their residues on rhizospheric and bulk soil eubacterial communities by means of denaturing gradient gel electrophoresis analyses of 16S rRNA genes, on the nontarget mycorrhizal symbiont Glomus mosseae, and on soil respiration. Microcosm experiments showed differences in rhizospheric eubacterial communities associated with the three corn lines and a significantly lower level of mycorrhizal colonization in Bt 176 corn roots. In greenhouse experiments, differences between Bt and non-Bt corn plants were detected in rhizospheric eubacterial communities (both total and active), in culturable rhizospheric heterotrophic bacteria, and in mycorrhizal colonization. Plant residues of transgenic plants, plowed under at harvest and kept mixed with soil for up to 4 months, affected soil respiration, bacterial communities, and mycorrhizal establishment by indigenous endophytes. The multimodal approach utilized in our work may be applied in long-term field studies aimed at monitoring the real hazard of genetically modified crops and their residues on nontarget soil microbial communities.

http://www.ncbi.nlm.nih.gov/pubmed/16269702

22. Flores S, Saxena D and Stotzky G. (2005): Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37: 10731082.

Bt plants are plants that have been genetically modified to express the insecticidal proteins (e.g. Cry1Ab, Cry1Ac, Cry3A) from subspecies of the bacterium, Bacillus thuringiensis (Bt), to kill lepidopteran pests that feed on corn, rice, tobacco, canola, and cotton and coleopteran pests that feed on potato. The biomass of these transgenic Bt plants (BtC) was decomposed less in soil than the biomass of their near-isogenic non-Bt plant counterparts (BtK). Soil was amended with 0.5, 1, or 2% (wt wtK1) ground, dried (50 8C) leaves or stems of Bt corn plants; with 0.5% (wt wtK1) ground, dried biomass of Bt rice, tobacco, canola, cotton, and potato plants; with biomass of the near-isogenic plants without the respective cry genes; or not amended. The gross metabolic activity of the soil was determined by CO2 evolution. The amounts of C evolved as CO2 were significantly lower from soil microcosms amended with biomass of Bt plants than of non-Bt plants. This difference occurred with stems and leaves from two hybrids of Bt corn, one of which had a higher C:N ratio than its near-isogenic non-Bt counterpart and the other which had essentially the same C:N ratio, even when glucose, nitrogen (NH4NO3), or glucose plus nitrogen were added with the biomass. The C:N ratios of the other Bt plants (including two other hybrids of Bt corn) and their near-isogenic non-Bt counterparts were also not related to their relative biodegradation. Bt corn had a significantly higher lignin content than near-isogenic non-Bt corn. However, the lignin content of the other Bt plants, which was significantly lower than that of both Bt and non-Bt corn, was generally not statistically significantly different, although 1066% higher, from that of their respective non-Bt near-isolines. The numbers of culturable bacteria and fungi and the activity of representative enzymes involved in the degradation of plant biomass were not significantly different between soil amended with biomass of Bt or non-Bt corn. The degradation of the biomass of all Bt plants in the absence of soil but inoculated with a microbial suspension from the same soil was also significantly less than that of their respective inoculated

non-Bt plants. The addition of streptomycin, cycloheximide, or both to the soil suspension did not alter the relative degradation of BtC and BtK biomass, suggesting that differences in the soil microbiota were not responsible for the differential decomposition of BtC and BtK biomass. All samples of soil amended with biomass of Bt plants were immunologically positive for the respective Cry proteins and toxic to the larvae of the tobacco hornworm (Manduca sexta), which was used as a representative lepidopteran in insect bioassays (no insecticidal assay was done for the Cry3A protein from potato). The ecological and environmental relevance of these findings is not clear.

23. Baumgarte S & Tebbe CC (2005): Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14 (8): 2539 2551.

Field studies were done to assess how much of the transgenic, insecticidal protein, Cry1Ab, encoded by a truncated cry1Ab gene fromBacillus thuringiensis (Bt), was released from Bt-maize MON810 into soil and whether bacterial communities inhabiting the rhizosphere of MON810 maize were different from those of the rhizosphere of nontransgenic maize cultivars. Bacterial community structure was investigated by SSCP (single-strand conformation polymorphism) of PCR-amplified 16S rRNA genes from community DNA. Using an improved extraction and detection protocol based on a commercially available ELISA, it was possible to detect Cry1Ab protein extracted from soils to a threshold concentration of 0.07 ng/g soil. From 100 ng of purified Cry1Ab protein added per gram of soil, only an average of 37% was extractable. At both field sites investigated, the amount of Cry1Ab protein in bulk soil of MON810 field plots was always lower than in the rhizosphere, the latter ranging from 0.1 to 10 ng/g soil. Immunoreactive Cry1Ab protein was also detected at 0.21 ng/g bulk soil 7 months after harvesting, i.e. in April of the following year. At this time, however, higher values were found in residues of leaves (21 ng/g) and of roots (183 ng/g), the latter corresponding to 12% of the Cry1Ab protein present in intact roots. A sampling 2 months later indicated further degradation of the protein. Despite the detection of Cry1Ab protein in the rhizosphere of MON810 maize, the bacterial community structure was less affected by the Cry1Ab protein than by other environmental factors, i.e. the age of the plants or field heterogeneities. The persistence of Cry1Ab protein emphasizes the importance of considering post-harvest effects on nontarget organisms.

http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2005.02592.x/Abstract

24. Kremer R.J, Means N.E and Kim S. (2005): Glyphosate affects soybean root exudation and rhizosphere microorganisms. Int. J. of Environmental Analytical Chemistry 85 (15): 1165-1174.

Glyphosate is a nonselective, broad-spectrum herbicide that kills plants by inhibiting the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), which is necessary for synthesis of aromatic amino acids. A secondary mode of action involves infection of roots by soilborne microorganisms due to decreased production of plant protection compounds known as phytoalexins. Varieties of several crops, including glyphosate-resistant (GR) or Roundup Ready soybean, are genetically modified to resist the herbicidal effects of glyphosate and provide farmers with an effective weed management tool. After glyphosate is applied to GR soybean, glyphosate that is not bound to glyphosate-resistant EPSPS is translocated throughout the plant and accumulates primarily in meristematic tissues. We previously reported that fungal colonization of GR soybean roots increased significantly after application of glyphosate but not after conventional postemergence herbicides. Because glyphosate may be released into soil from GR roots, we characterized the response of rhizosphere fungi and bacteria to root exudates from GR and non-GR cultivars treated with and without glyphosate at field application rates. Using an immunoassay technique, the flux of glyphosate detected in exudates of hydroponically grown GR soybean was > 1000 ng plant**-1 over the 16-d post-glyphosate application period. Glyphosate also increased carbohydrate and amino acid contents in root exudates in both soybean cultivars. However, GR soybean released higher carbohydrate and amino acid contents in root exudates than W82 soybean without glyphosate treatment. In vitro bioassays showed that glyphosate in the exudates stimulated growth of selected rhizosphere fungi, possibly by providing a selective C and N source combined with the high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of the soybean plants. Increased fungal populations that develop under glyphosate treatment of GR soybean may adversely affect plant growth and biological processes in the soil and rhizosphere.

http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=170287

25. Griffiths B.S, Caul S, Thompson J, Birch A.N.E, Scrimgeour C, Andersen M.N, Cortet J, Messéan A, Sausse C, Lacroix B. & Krogh P.H. (2005): A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the Bacillus thuringiensis CryIAb toxin. Plant and Soil 275: 135-146.

Field trials were established at three European sites (Denmark, Eastern France, South-West France) of genetically modified maize (Zea mays L.) expressing the CryIAb Bacillus thuringiensis toxin (Bt), the near-isogenic non-Bt cultivar, another conventional maize cultivar and grass. Soil from Denmark was sampled at sowing (May) and harvest (October) over two years (2002, 2003); from E France at harvest 2002, sowing and harvest 2003; and from SW France at sowing and harvest 2003. Samples were analysed for microbial community structure (2003 samples only) by community-level physiological-profiling (CLPP) and phospholipid fatty acid analysis (PLFA), and protozoa and nematodes in all samples. Individual differences within a site resulted from: greater nematode numbers under grass than maize on three occasions; different nematode populations under the conventional maize cultivars once; and two occasions when there was a reduced protozoan population under Bt maize compared to non-Bt maize. Microbial community structure within the sites only varied with grass compared to maize, with one occurrence of CLPP varying between maize cultivars (Bt versus a conventional cultivar). An overall comparison of Bt versus non-Bt maize across all three sites only revealed differences for nematodes, with a smaller population under the Bt maize. Nematode community structure was different at each site and the Bt effect was not confined to specific nematode taxa. The effect of the Bt maize was small and within the normal variation expected in these agricultural systems.

http://www.ingentaconnect.com/content/klu/plso/2005/00000275/f0020001/00001093

26. Saxena D, Stewart C N, Altosaar I, Shu Q & Stotzky G (2004): Larvicidal Cry proteins from Bacillus thuringiensis are released in root exudates of transgenic B. thuringiensis corn, potato, and rice but not of B. thuringiensis canola, cotton, and tobacco. Plant Physiology & Biochemistry 42 (5): 383387.

Larvicidal proteins encoded by cry genes from Bacillus thuringiensis were released in root exudates from transgenic B. thuringiensis corn, rice, and potato but not from B. thuringiensis canola, cotton, and tobacco. Nonsterile soil and sterile hydroponic solution in which B. thuringiensis corn, rice, or potato had been grown were immunologically positive for the presence of the Cry proteins; from B. thuringiensis corn and rice, the soil and solution were toxic to the larva of the tobacco hornworm (Manduca sexta), and from potato, to the larva of the Colorado potato beetle (Leptinotarsa decemlineata), representative lepidoptera and coleoptera, respectively. No toxin was detected immunologically or by larvicidal assay in soil or hydroponic solution in which B. thuringiensis canola, cotton, or tobacco, as well as all near-isogenic non-B. thuringiensis plant counterparts or no plants, had been grown. All plant species had the cauliflower mosaic virus (CaMV) 35S promoter, except rice, which had the ubiquitin promoter from maize. The reasons for the differences between species in the exudation from roots of the toxins are not known. The released toxins persisted in soil as the result of their binding on surface-active particles (e.g. clay minerals, humic substances), which reduced their biodegradation. The release of the toxins in root exudates could enhance the control of target insect pests, constitute a hazard to nontarget organisms, and/or increase the selection of toxin-resistant target insects.

http://www.ncbi.nlm.nih.gov/pubmed/15191740

27. Wei-Xiang Wu (2004): Effect of straws from Bt transgenic rice on selected biological activities in water-flooded soil. European Jounral of Soil Biology 40 (1): 15-22.

The biochemical properties of soil have often been described as early and sensitive indicators of ecological changes in both natural soil and agroecosystem. In the current study, the impacts of the amendment of Bt-transgenic rice (KMD) straw on biological activities in water-flooded soil were investigated under laboratory conditions and compared with non-transgenic rice (Xiushui 11) straw. The results showed that there were some differences in protease, neutral phosphatase and cellulase activities between soil amended with Bt-transgenic rice straw and non-transgenic rice straw at the early stage of incubation, and none of these differences were persistent. However, differences in dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration between soil supplemented with Bt-transgenic rice straw and non-transgenic rice straw were persistent over the course of incubation. Dehydrogenase activity, methanogenesis and anaerobic respiration were considerably lower from sample days 7 to 56, but higher after day 56 in soil amended with Bt-transgenic rice straw. In comparison, the H2-production in soil containing Bt-transgenic rice straw was significantly lower after day 56. The results demonstrated that the amendment of the Bt-transgenic rice straw altered some important biological properties in water-flooded soil, indicating a shift in microbial populations or a change in the metabolic abilities of the microbial community as a result of substrate availability in soil.

http://journals2.scholarsportal.info/details.xqy?uri=/11645563/v40i0001/15_eosfbrsbaiws.xml

28. Dunfield K E and Germida J J (2004): Impact of genetically modified crops on soiland plant-associated microbial communities. J. Environ. Qual. 33 (3), 806815.

Transgenic or genetically modified plants possess novel genes that impart beneficial characteristics such as herbicide resistance. One of the least understood areas in the environmental risk assessment of genetically modified crops is their impact on soil- and plant-associated microbial communities. The potential for interaction between transgenic plants and plant residues and the soil microbial community is not well understood. The recognition that these interactions could change microbial biodiversity and affect ecosystem functioning has initiated a limited number of studies in the area. At this time, studies have shown the possibility that transgenes can be transferred to native soil microorganisms through horizontal gene transfer, although there is not evidence of this occurring in the soil. Furthermore, novel proteins have been shown to be released from transgenic plants into the soil ecosystem, and their presence can influence the biodiversity of the microbial community by selectively stimulating the growth of organisms that can use them. Microbial diversity can be altered when associated with transgenic plants; however, these effects are both variable and transient. Soil- and plant-associated microbial communities are influenced not only by plant species and transgene insertion but also by environmental factors such as field site and sampling date. Minor alterations in the diversity of the microbial community could affect soil health and ecosystem functioning, and therefore, the impact that plant variety may have on the dynamics of the rhizosphere microbial populations and in turn plant growth and health and ecosystem sustainability, requires further study.

https://www.crops.org/publications/jeq/Abstracts/33/3/0806?access=0&view=pdf

29. Stotzky G. (2004): Persistence and biological activity in soil of the insecticidal proteins from Bacillus thuringiensis, especially from transgenic plants. Plant and Soil 266: 77-89.

Insecticidal proteins produced by various subspecies (kurstaki, tenebrionis, and israelensis) of Bacillus thuringiensis (Bt) bound rapidly and tightly on clays, both pure mined clay minerals and soil clays, on humic acids extracted from soil, and on complexes of clay and humic acids. Binding reduced susceptibility of the proteins to microbial degradation. However, bound proteins retained biological activity. Purified Cry1Ab protein and protein released from biomass of transgenic Bt corn and in root exudates of growing Bt corn (13 hybrids representing three transformation events) exhibited binding and persistence in soil. Insecticidal protein was also released in root exudates of Bt potato (Cry3A protein) and rice (Cry1Ab protein) but not in root exudates of Bt canola, cotton, and tobacco (Cry1Ac protein). Vertical movement of CrylAb protein, either purified or in root exudates or biomass of Bt corn, decreased as the concentration of the clay minerals, kaolinite or montmorillonite, in soil increased. Biomass of transgenic Bt corn decomposed less in soil than biomass of near-isogenic non-Bt corn, possibly because biomass of Bt corn had a significantly higher content of lignin than biomass of non-Bt corn. Biomass of Bt canola, cotton, potato, rice, and tobacco also decomposed less than biomass of the respective near-isogenic non-Bt plants. However, the lignin content of these Bt plants, which was significantly less than that of Bt corn, was not significantly different from that of their near-isogenic non-Bt counterparts, although it was consistently higher. The Cry1Ab protein had no consistent effects on organisms (earthworms, nematodes, protozoa, bacteria, fungi) in soil or in vitro. The Cry1Ab protein was not taken up from soil by non-Bt corn, carrot, radish, or turnip grown in soil in which Bt corn had been grown or into which biomass of Bt corn had been incorporated.

30. Motavalli P P, Kremer R J, Fang M and Means N E (2004): Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. J. Environ. Qual. 33 (3), 81624.

One of the potential environmental effects of the recent rapid increase in the global agricultural area cultivated with transgenic crops is a change in soil microbially mediated processes and functions. Among the many essential functions of soil biota are soil organic matter decomposition, nutrient mineralization and immobilization, oxidation-reduction reactions, biological N fixation, and solubilization. However, relatively little research has examined the direct and indirect effects of transgenic crops and their management on microbially mediated nutrient transformations in soils. The objectives of this paper are to review the available literature related to the environmental effects of transgenic crops and their management on soil microbially mediated nutrient transformations, and to consider soil properties and climatic factors that may affect the impact of transgenic crops on these processes. Targeted genetic traits for improved plant nutrition include greater plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N. Among the potential direct effects of transgenic crops and their management are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources. Possible indirect effects of transgenic crops, including changes in the fate of transgenic crop residues and alterations in land use and rates of soil erosion, deserve further study. Despite widespread public concern, no conclusive evidence has yet been presented that currently released transgenic crops, including both herbicide and pest resistant crops, are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments. Further consideration of the effects of a wide range of soil properties, including the amount of clay and its mineralogy, pH, soil structure, and soil organic matter, and variations in climatic conditions, under which transgenic crops may be grown, is needed in evaluating the impact of transgenic crops on soil nutrient transformations. Future environmental evaluation of the impact of the diverse transgenic crops under development could lead to an improved understanding of soil biological functions and processes.

31. Zwahlen C, Hilbeck A, Howald R and Nentwig W. (2003): Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12 (8):1077 1086.

A 200-day study was carried out to investigate the impact of transgenic Bacillus thuringiensis (Bt) corn on immature and adult Lumbricus terrestris in the field and in the laboratory. Another objective of this study was to develop test methods that could be used for standard testing of the impact of transgenic plants on different earthworm species in the field and in the laboratory. For this purpose two different experiments were involved, a laboratory experiment with adult L. terrestris and a field experiment with immature L. terrestris. No lethal effects of transgenic Bt corn on immature and adult earthworms were observed. Immature L. terrestris in the field had a very similar growth pattern when fed either (Bt+) or (Bt) corn litter. No significant differences in relative weights of (Bt+) and (Bt) corn-fed adult L. terrestris were observed during the first 160 days of the laboratory trial, but after 200 days adult L. terrestris had a significant weight loss of 18% of their initial weight when fed (Bt+) corn litter compared to a weight gain of 4% of the initial weight of (Bt) corn-fed earthworms. Further studies are necessary to see whether or not this difference in relative weight was due to the Bt toxin or other factors discussed in the study. Degradation of Cry1Ab toxin in corn residues was significantly slower in the field than at 10 C in the laboratory. Enzyme-linked immunosorbent assay results indicated that earthworms in both experiments were exposed to the Bt toxin throughout the whole experimental time.

32. Zwahlen C, Hilbeck A, Gugerli P & Nentwig W. (2003): Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Molecular Ecology 12 (3): 765-775.

Large quantities of Bacillus thuringiensis (Bt) corn plant residue are left in the field after harvest, which may have implications for the soil ecosystem. Potential impacts on soil organisms will also depend on the persistence of the Bt toxin in plant residues. herefore, it is important to know how long the toxin persists in plant residues. In two field studies in the temperate corn-growing region of Switzerland we investigated degradation of the Cry1Ab toxin in transgenic Bt corn leaves during autumn, winter and spring using an enzyme-linked immunosorbent assay (ELISA). In the first field trial, representing a tillage system, no degradation of the Cry1Ab toxin was observed during the first month. During the second month, Cry1Ab toxin concentrations decreased to 20% of their initial values. During winter, there was no further degradation. When temperatures again increased in spring, the toxin continued to degrade slowly, but could still be detected in June. In the second field trial, representing a no-tillage system, Cry1Ab toxin concentrations decreased without initial delay as for soil-incorporated Bt plants, to 38% of the initial concentration during the first 40 days. They then continued to decrease until the end of the trial after 200 days in June, when 0.3% of the initial amount of Cry1Ab toxin was detected. Our results suggest that extended pre- and post-commercial monitoring are necessary to assess the long-term impact of Bt toxin in transgenic plant residues on soil organisms.

http://4ccr.pgr.mpf.gov.br/institucional/grupos-de-trabalho/gt transgenicos/bibliografia/pgm-e-riscosambientais/Zwahlen%20et%20al,%202003,%20Mol%20Ecolo.pdf

33. Sun C, Wu Z, Zhang Y & Zhang L (2003): Effect of transgenic Bt rice planting on soil enzyme activities. Ying Yong Sgeng Tai Xue Bao 14: 2261-2264.

A pot experiment was conducted with silty loam Agrodolf as test soil and with transgenic Bt rice and non-Bt rice as test crops to study the effect of transgenic Bt rice planting on soil urease, phophatase,arylsulfatase, invertase, and dehydrogenase activities. The results showed that Bt toxin could be introduced into soil through root exudates of transgenic Bt rice, and its survival amount in soil varied with time. Compared with non-Bt rice treatment, transgenic Bt rice treatment had a significant decrease (2.47%) of soil urease activity and a significant increase (8.91%) of soil acid phosphataseactivity, but no significant change in soil arylsulfatase, invertase, and dehydrogenase activities at the 15th day of emergence. At the 30th day of emergence, the transgenic Bt rice treatment still had a significant decrease of soil urease activity (16.36%) and a significant increase of acid phosphataseactivity (35.69%), and no change in invertase activity. It also had significant increase in soilarylsulfatase (19.70%) and dehydrogenase activities (16.83%).

http://europepmc.org/Abstract/MED/15031930/reload=0;jsessionid=HSzLydStrsWxwir5XMLX.4

34. Saxena D., Flores S. & Stotzky G. (2002): Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology and Biochemistry 34: 133-137.

Cry 1Ab Bt protein from 3 different transformation events was detected in root exudates and rhizosphere of seedlings grown for 40 days in the lab and also grown in the field. Protein detection was achieved using Quickstix ELISA and bioassay (mortality was between 38 and 100%).

35. Bruinsma M, Kowalchuk GA and van Veen JA (2003): Effects of genetically modified plants on microbial communities and processes in soil. Biol. Fertil. Soils 37: 329-337

The development and use of genetically modified plants (GMPs) has been a topic of considerable public debate in recent years. GMPs hold great promise for improving agricultural output, but the potential for unwanted effects of GMP use is still not fully understood. The majority of studies addressing potential risks of GMP cultivation have addressed only aboveground effects. However, recent methodological advances in soil microbial ecology have allowed research focus to move underground to try to gain knowledge of GMP-driven effects on the microbial communities and processes in soil that are essential to key terrestrial ecosystem functions. This review gives an overview of the research performed to date on this timely topic, highlighting a number of case studies. Although such research has advanced our understanding of this topic, a number of knowledge gaps still prevent full interpretation of results, as highlighted by the failure of most studies to assign a definitively negative, positive or neutral effect to GMP introduction. Based upon our accumulating, yet incomplete, understanding of soil microbes and processes, we propose a synthesis for the case-by-case study of GMP effects, incorporating assessment of the potential plant/ecosystem interactions, accessible and relevant indicators, and tests for unforeseen effects.

36. Reddy K.N. and Zablotowicz R.M. (2003): Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. Weed Science 51 (4): 496502.

A field study was conducted during 2000 and 2001 at Stoneville, MS, to determine the effects of isopropylamine, trimethylsulfonium (Tms), diammonium, and aminomethanamide dihydrogen tetraoxosulfate (Adt) salt formulations of glyphosate on weed control, growth, chlorophyll content, nodulation, nitrogen content, and grain yield in glyphosate-resistant soybean and to assess potential glyphosate accumulation in soybean nodules. Glyphosate-Tms and glyphosate-Adt injured soybean, and visible injury ranged from 29 to 38% 2 d after late postemergence (LPOST) application; however, soybean recovered by 14 d. Glyphosate formulations had no effect on chlorophyll content, root and shoot dry weight, or nodule number but reduced nodule biomass by 21 to 28% 14 d LPOST. Glyphosate levels in nodules from treated plants ranged from 39 to 147 ng g21 (dry weight), and leghemoglobin content was reduced by as much as 10%. Control of five predominant weed species 14 d after LPOST was . 83% with one application and . 96% with two applications regardless of the glyphosate salts used. Soybean yields were generally higher with two applications than with one application regardless of glyphosate formulation. These results indicate that soybean injury and inhibition of nodule development with certain glyphosate formulations can occur, but soybean has the potential to recover from glyphosate stress.

http://naldc.nal.usda.gov/download/48637/PDF

37. King CA, Purcell LC and Vories ED. (2001): Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. AgronomyJournal 93 (1), 179186.

Glyphosate [N-(phosphonomethyl)glycine] inhibits 5-enolpyruvylshikimate-3-phosphate synthase, EC 2.5.1.19 (EPSPS), thereby blocking aromatic amino acid synthesis. While glyphosate-tolerant (GT) soybean [Glycine max (L.) Merr.] contains resistant EPSPS, the N2-fixing symbiont in soybean root nodules, Bradyrhizobium japonicum, does not contain a resistant enzyme, and glyphosate spray to GT soybean may interfere with the symbiotic relationship. Glyphosate-tolerant soybean was treated with glyphosate at several different stages of development to evaluate N2 fixation, growth, and yield in a series of greenhouse, growth chamber, and field experiments. Early applications of glyphosate generally delayed N2 fixation and decreased biomass and N accumulation in the cultivar Terral TV5866RR (TV5866RR) harvested at 19 d after emergence (DAE), but plants had recovered by 40 DAE. The biomass and N content of GT soybean were also decreased by glyphosate in plants that were grown with available soil N. There were differences in sensitivity to glyphosate among GT cultivars, with biomass decreases in response to glyphosate ranging from 0 to 30% at 40 DAE for the most tolerant and sensitive cultivars that were evaluated. In growth chamber studies, N2 fixation was more sensitive to water deficits in glyphosate-treated plants. In field studies, there was no measured effect of glyphosate on GT soybean at Fayetteville, AR where there was adequate soil water throughout the growing season. However, glyphosate tended to decrease biomass and seed yields under conditions of limited soil water.

https://www.crops.org/publications/aj/Abstracts/93/1/179?access=0&view=pdf

38. Sanogo S, Yang, X., Scherm, H. (2000): Effects of herbicides on Fusarium solani f. sp. glycines and development of sudden death syndrome in glyphosate-tolerant soybean. Phytopathology 90: 5766.

Sudden death syndrome of soybean, caused by Fusarium solani f. sp. glycines, is a disease of increasing economic importance in the United States. Although the ecology of sudden death syndrome has been extensively studied in relation to crop management practices such as tillage, irrigation, and cultivar selection, there is no information on the effects of herbicides on this disease. Three herbicides (lactofen, glyphosate, and imazethapyr) commonly used in soybean were evaluated for their effects on the phenology of F. solani f. sp. glycines and the development of sudden death syndrome in four soybean cultivars varying in resistance to the disease and in tolerance to glyphosate. Conidial germination, mycelial growth, and sporulation in vitro were reduced by glyphosate and lactofen. In growth-chamber and greenhouse experiments, there was a significant increase in disease severity and frequency of isolation of F. solani f. sp. glycines from roots of all cultivars after application of imazethapyr or glyphosate compared with the control treatment (no herbicide applied). Conversely, disease severity and isolation frequency of F. solani f. sp. glycines decreased after application of lactofen. Across all herbicide treatments, severity of sudden death syndrome and isolation frequency were lower in disease-resistant than in susceptible cultivars. Results suggest that glyphosate-tolerant and -nontolerant cultivars respond similarly to infection by F. solani f. sp. glycines after herbicide application.

http://www.ncbi.nlm.nih.gov/pubmed/18944572

39. University of Missouri (2000): MU researchers find fungi buildup in glyphosate-treated soybean fields. University of Missouri, 21 December.

Increased and frequent use of glyphosate associated with Roundup Ready (RR) soybean production can affect activities of rhizosphere and soil microorganisms. Glyphosate influence on interactions of soybean with soybean cyst nematode (SCN; Heterodera glycines) and rhizosphere fungi may have potential implication in future management. Field experiments were conducted to determine the impact of glyphosate applied to RR soybean on root and soil colonization by Fusarium spp. and SCN. In 1997 and 1998, RR soybean receiving glyphosate at 1X and 3X recommended rate had significantly higher incidence of Fusarium on roots compared with control (no glyphosate) at one Missouri site. In 1999, glyphosate, conventional herbicide mix (pendimethalin+imazaquin), and glyphosate+conventional were evaluated on four RR soybean varieties at eight sites. Frequency of Fusarium on roots increased 0.5 - 5X at 2 or 4 wk after application of glyphosate or glyphosate+conventional herbicides compared with the conventional herbicide alone. Soil Fusarium populations varied among sites. Effects on SCN reproduction were variable. Increased Fusarium colonization of RR soybean roots with glyphosate application may influence potential disease level.

www.biotechinfo.net/fungi_buildup.html

40. Saxena D, Flores S and Stozsky G (1999): Insecticidal toxin in root exudates from Bt corn. Nature 402: 480.

Seeds of Bt (NK4640Bt) and isogenic corn were germinated on agar. Seedlings were placed on plastic screening suspended over Hoaglands solution. Soil free medium was removed and analyzed after 7, 15 and 25 days of growth at which time it was replaced with fresh solution. Cry1Ab protein was found after 7 and 15 days in the exudates from Bt corn and exhibited insecticidal activity. Cry1Ab protein was not detected after 25 days, when the medium was no longer sterile. Supernatants of rhizosphere soil of seedlings transplanted in sterile or non-sterile soil was analyzed using immunological and larvicidal assays. These tests were positive for Bt corn and negative for non-Bt corn after 25 days of growth.

http://www.ent.iastate.edu/toxicology/node/230

41. Tapp H and Stozsky G (1998): Persistence of the insecticidal toxin from Bacillus thuringiensis subsp kurstaki in soil. Soil Biol. Biochem. 30 (4): 471-476.

The accumulation and persistence of the insecticidal toxins from Bacillus thuringiensis may result in environmental hazards, such as toxicity to nontarget species and the selection of toxin-resistant target species. Toxins from B. thuringiensis subsp. kurstaki were added to three soils [Kitchawan soil (which contains kaolinite but not montmorillonite) unamended or amended with montmorillonite or kaolinite (as an internal control); Mopala soil, which contains montmorillonite and kaolinite; and San Alejo soil, which does not contain montmorillonite but contains kaolinite], and the persistence of the toxins was determined by insect bioassays using the larvae of the tobacco hornworm (Manduca sexta). Toxicity varied with the type of soil: the Kitchawan soil, either unamended or amended with kaolinite, remained toxic to the larvae for more than 6 months, maintaining a lethal concentration at which 50% of the larvae were killed (LC50) of 61 to 111 ng 100 microliter-1 of soil suspension throughout 195 d of incubation. The Kitchawan soil amended with montmorillonite and the Mopala and San Alejo soils showed reduced insecticidal activity after only 35 d (LC50 from 104 to 192 ng 100 microliter-1). The pH of soils in which insecticidal activity was reduced was higher (5.8 to 7.3) than that of soils in which insecticidal activity was retained (4.9 to 5.1). As microbial activity is greater at higher pH values, more of the toxins may have been degraded by microbes in soils with the higher pH values. This hypothesis was confirmed by the greater loss in insecticidal activity during 234 d when the pH of the Kitchawan soil, unamended or amended to 6% (vol vol-1) with kaolinite, was increased from 4.9 to ca. 7.0 by the addition of CaCO3.

http://europepmc.org/abstract/AGR/IND21958436

HORIZONTAL GENE TRANSFER

1. Zhang L, Hou D, Chen X, et al. (2012): Exogenous plant MIR168a specifically targets mammalian LDLRAP1: Evidence of cross-kingdom regulation by microRNA. Cell Res. 22(1):107-26 (Erratum in: Cell Res. 2012 Jan;22(1):273-4)

Our previous studies have demonstrated that stable microRNAs (miRNAs) in mammalian serum and plasma are actively secreted from tissues and cells and can serve as a novel class of biomarkers for diseases, and act as signaling molecules in intercellular communication. Here, we report the surprising finding that exogenous plant miRNAs are present in the sera and tissues of various animals and that these exogenous plant miRNAs are primarily acquired orally, through food intake. MIR168a is abundant in rice and is one of the most highly enriched exogenous plant miRNAs in the sera of Chinese subjects. Functional studies in vitro and in vivo demonstrated that MIR168a could bind to the human/mouse low-density lipoprotein receptor adapter protein 1 (LDLRAP1) mRNA, inhibit LDLRAP1 expression in liver, and consequently decrease LDL removal from mouse plasma. These findings demonstrate that exogenous plant miRNAs in food can regulate the expression of target genes in mammals.

2. Chen J, Jin M, Qiu ZG, Guo C, Chen ZL, Shen ZQ, Wang XW, Li JW. (2012): A survey of drug resistance bla genes originating from synthetic plasmid vectors in six Chinese rivers Environ Sci Technol. 46(24):13448-54. doi: 10.1021/es302760s.

Antibiotic resistance poses a significant challenge to human health and its rate continues to rise globally. While antibiotic-selectable synthetic plasmid vectors have proved invaluable tools of genetic engineering, this class of artificial recombinant DNA sequences with high expression of antibiotic resistance genes presents an unknown risk beyond the laboratory setting. Contamination of environmental microbes with synthetic plasmid vector-sourced antibiotic resistance genes may represent a yet unrecognized source of antibiotic resistance. In this study, PCR and real-time quantitative PCR were used to investigate the synthetic plasmid vector-originated ampicillin resistance gene, ?-lactam antibiotic (blá), in microbes from six Chinese rivers with significant human interactions. Various levels of blá were detected in all six rivers, with the highest levels in the Pearl and Haihe rivers. To validate the blá pollution, environmental plasmids in the river samples were captured by the E. coli transformants from the community plasmid metagenome. The resultant plasmid library of 205 ampicillin-resistant E. coli (transformants) showed a blá-positive rate of 27.3% by PCR. Sequencing results confirmed the synthetic plasmid vector sources. In addition, results of the Kirby-Bauer disc-diffusion test reinforced the ampicillin-resistant functions of the environmental plasmids. The resistance spectrum of transformants from the Pearl and Haihe rivers, in particular, had expanded to the third- and fourth-generation of cephalosporin drugs, while that of other transformants mainly involved first- and second-generation cephalosporins. This study not only reveals environmental contamination of synthetic plasmid vector-sourced blá drug resistance genes in Chinese rivers, but also suggests that synthetic plasmid vectors may represent a source of antibiotic resistance in humans.

3. Dana CE, Glauber KM, Chan TA, Bridge DM, Steele RE (2012): Incorporation of a Horizontally Transferred Gene into an Operon during Cnidarian Evolution. PLoS ONE 7(2): e31643

Genome sequencing has revealed examples of horizontally transferred genes, but we still know little about how such genes are incorporated into their host genomes. We have previously reported the identification of a gene (flp) that appears to have entered the Hydra genome through horizontal transfer. Here we provide additional evidence in support of our original hypothesis that the transfer was from a unicellular organism, and we show that the transfer occurred in an ancestor of two medusozoan cnidarian species. In addition we show that the gene is part of a bicistronic operon in the Hydra genome. These findings identify a new animal phylum in which trans-spliced leader addition has led to the formation of operons, and define the requirements for evolution of an operon in Hydra. The identification of operons in Hydra also provides a tool that can be exploited in the construction of transgenic Hydra strains.

4. Douville M, Gagne F, André C and Blaise C. (2009): Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (Elliptio complanata) near cornfields: evidence of exposure by bacterial ingestion. Ecotoxicology and Environmental Safety 72: 1725

The purpose of this study was to examine the contamination of cry1 and cry1Ab genes from Bacillus thuringiensis and transgenic corn in feral freshwater mussels collected from sites located in proximity of corn fields. In addition, mussels were transplanted for 2 months to a site in the Huron River, upstream to the Richelieu River, which is subject to intensive corn farming. Mussels were significantly contaminated by both genes in their gills, digestive glands, and gonads, as determined by qPCR methodology. Gene sequence analysis confirmed the presence of transgenic corn cry1Ab gene in mussel tissues. In an attempt to explain the presence of the transgene in mussel tissues, heterotrophic bacteria were grown from surface water and sediment samples on agar plates in the Richelieu River in May and August. The transgene was found at two out of six surface water samples and in one sediment sample. The study revealed that exposure to transgenic corn cry1Ab gene in mussels seems to proceed by ingestion of microorganisms during feeding.

http://www.sciencedirect.com/science/article/pii/S0147651308000493

5. Ran T, Mei L, Lei W, Aihua L, Ru H, Jie S. (2009): Detection of transgenic DNA in tilapias (Oreochromis niloticus, GIFT strain) fed genetically modified soybeans (Roundup Ready). Aquaculture Research. 40: 13501357

We used nested-polymerase chain reaction (PCR) to detect Roundup Ready soybean in aquatic feeds and feeding tilapias. A template concentration of 10−10 g μL−1 DNA solution could be detected with a dilute degree of 0.01%. Most (90.6%) of the aquatic feeds containing soybean byproduct included exogenous DNA segments. We also compared genetically modified (GM) soybean with non-GM soybean diets in feeding tilapias (Oreochromis niloticus, GIFT strain) and examined the residual fragments (254 bp) of GM soybeans. Tilapias receiving GM soybean diets had DNA fragments in different tissues and organs, indicating that exogenous GM genes were absorbed systemically and not completely degraded by the tilapia's alimentary canal.

6. Chainark P, Satoh S, Hirono I, Aoki T, Endo M. (2008): Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout Oncorhynchus mykiss. Fisheries Science. 74: 380390

Foreign DNA fragments from genetically modified defatted soybean meal (GM SBM) in rainbow trout was traced by nested polymerase chain reaction (PCR) and located by in situ hybridization. Either a GM or non-GM SBM formulated diet (42% protein) was fed to fish (average weight 50.5 g) for 2 weeks. The degradation results showed that the cauliflower mosaic virus 35S promoter (220 bp) fragment was detected in the contents of digestive system only in fish fed the GM SBM diet, and it was not detected on the third day after changing the diet to the non-GM SBM diet. For the possible transferal results, the promoter fragment was detected in the leukocyte, head kidney and muscle only of fish fed the GM SBM diet; it was not detected on the fifth day after changing the diet to the non-GM SBM diet. These results suggest that a foreign DNA fragment was not completely degraded and might be taken up into organs through the gastrointestinal tract. However, foreign DNA was not detected after the withdrawal period. Thus, the data show that uptake of DNA from GM SBM might not remain in the tissues of fish fed GM SBM diet.

7. Tudisco R, Infascelli F, Cutrignelli M I, Bovera F, Morcia C, Facciolli P and Terzi V (2006): Fate of feed plant DNA monitored in water buffalo (Bubalus bubalis) and rabbit (Oryctolagus cuniculus). Livestock Science Vol. 105 (1-3): 12-18.

The effect of the digestion process in the gastro-intestinal tract (GIT) of animal models on the fate and integrity of plant DNA has been widely evaluated since DNA availability and integrity is a key factor for hypothetical horizontal gene transfer of recombinant DNA from GM crop-derived feeds to animal and human gut microflora. In this study, plant DNA sequences from high and low copy number genes were monitored in GIT and tissues of buffaloes and rabbits. Using a real-time PCR approach to track plant DNA in animal samples, we demonstrated the persistence of fragmented plant DNA blood and tissues of buffaloes and rabbits raised with conventional feeding.

http://www.livestockscience.com/article/S1871-1413(06)00227-7/Abstract

8. Smets BF and Barkay T (2005): Horizontal gene transfer: perspectives at a crossroads of scientific disciplines. Nature Reviews Microbiology 3: 675-678.

Horizontal gene transfer (HGT) has a crucial role in microbial evolution, in shaping the structure and function of microbial communities and in controlling a myriad of environmental and public-health problems. Here, Barth F. Smets and Tamar Barkay assess the importance of HGT and place the selection of articles in this Focus issue in context.

http://www.nature.com/nrmicro/journal/v3/n9/full/nrmicro1253.html

9. Woloszynska M, Bocer T, Mackiewicz P and Janska H (2004): A fragment of chloroplast DNA was transferred horizontally, probably from non-eudicots, to mitochondrial genome of Phaseolus. Plant Mol Biol. 56 (5): 811-20

The mitochondrial genomes of some Phaseolus species contain a fragment of chloroplast trnA gene intron, named pvs-trnA for its location within the Phaseolus vulgaris sterility sequence (pvs). The purpose of this study was to determine the type of transfer (intracellular or horizontal) that gave rise to pvs-trnA. Using a PCR approach we could not find the respective portion of the trnA gene as a part of pvs outside the Phaseolus genus. However, a BLAST search revealed longer fragments of trnA present in the mitochondrial genomes of some Citrus species, Helianthus annuus and Zea mays. Basing on the identity or near-identity between these mitochondrial sequences and their chloroplast counterparts we concluded that they had relocated from chloroplasts to mitochondria via recent, independent, intracellular DNA transfers. In contrast, pvs-trnA displayed a relatively higher sequence divergence when compared with its chloroplast counterpart from Phaseolus vulgaris. Alignment of pvs-trnA with corresponding trnA fragments from 35 plant species as well as phylogenetic analysis revealed that pvs-trnA grouped with non-eudicot sequences and was well separated from all Fabales sequences. In conclusion, we propose that pvs-trnA arose via horizontal transfer of a trnA intron fragment from chloroplast of a non-eudicot plant to Phaseolus mitochondria. This is the first example of horizontal transfer of a chloroplast sequence to the mitochondrial genome in higher plants.

http://www.ncbi.nlm.nih.gov/pubmed/15803417

10. Daubin V and Ochman H (2004): Quartet mapping and the extent of lateral transfer in bacterial genomes. Mol Biol Evol 21 (1): 86-89.

Several recent analyses have used quartet-based methods to assess the congruence among phylogenies derived for large sets of genes from prokaryotic genomes. The principal conclusion from these studies is that lateral gene transfer (LGT) has blurred prokaryotic phylogenies to such a degree that the darwinian scheme of treelike evolution might be abandoned in favor of a net or web. Here, we focus on one of these methods, quartet mapping, and show that its application can lead to overestimation of the extent of inferred LGT in prokaryotes, particularly when applied to distantly related taxa.

http://mbe.oxfordjournals.org/content/21/1/86.full.pdf

11. Steale RE, Hampson SE, Stover NA, Kibler DF and Bode HR (2004): Probable horizontal transfer between a protist and a cnidarian. Curr Biol 14(8): 298-9.

12. Davis CC and Wurdack KJ (2004): Host-to-parasite gene transfer in flowering plants: Phylogenetic evidence from Malpighiales. Science 305: 676-78.

Horizontal gene transfer (HGT) between sexually unrelated species has recently been documented for higher plants, but mechanistic explanations for HGTs have remained speculative. We show that a parasitic relationship may facilitate HGT between flowering plants. The endophytic parasites Rafflesiaceae are placed in the diverse order Malpighiales. Our multigene phylogenetic analyses of Malpighiales show that mitochrodrial (matR) and nuclear loci (18S ribosomal DNA and PHYC) place Rafflesiaceae in Malpighiales, perhaps near Ochnaceae/ Clusiaceae. Mitochondrial nad1B-C, however, groups them within Vitaceae, near their obligate host Tetrastigma. These discordant phylogenetic hypotheses strongly suggest that part of the mitochondrial genome in Rafflesiaceae was acquired via HGT from their hosts.

http://www.people.fas.harvard.edu/~ccdavis/pdfs/Davis_Wurdack_Science_2004.pdf

13. Sharma R, Alexander TW, John SJ, Forster RJ and McAllister TA (2004): Relative stability of transgene DNA fragments from GM rapeseed in mixed ruminal cultures. British Journal of Nutrition 91 (5): 673-681.

The use of transgenic crops as feeds for ruminant animals has prompted study of the possible uptake of transgene fragments by ruminal micro-organisms and/or intestinal absorption of fragments surviving passage through the rumen. The persistence in buffered ruminal contents of seven different recombinant DNA fragments from GM rapeseed expressing the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) transgene was tracked using PCR. Parental and transgenic (i.e. glyphosphate-tolerant; Roundup Ready, Monsanto Company, St Louis, MO, USA) rapeseed were incubated for 0, 2, 4, 8, 12, 24 and 48 h as whole seeds, cracked seeds, rapeseed meal, and as pelleted, barley-based diets containing 65 g rapeseed meal/kg. The seven transgene fragments ranged from 179 to 527 bp and spanned the entire 1363 bp EPSPS transgene. A 180 bp ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit fragment and a 466 bp 16S rDNA fragment were used as controls for endogenous rapeseed DNA and bacterial DNA respectively. The limit of detection of the PCR assay, established using negative controls spiked with known quantities of DNA, was 12.5 pg. Production of gas and NH3 was monitored throughout the incubation and confirmed active in vitro fermentation. Bacterial DNA was detected in all sample types at all time points. Persistence patterns of endogenous (Rubisco) and recombinant (EPSPS) rapeseed DNA were inversely related to substrate digestibility (amplifiable for 48, 8 and 4 h in whole or cracked seeds, meal and diets respectively), but did not differ between parental and GM rapeseed, nor among fragments. Detection of fragments was representative of persistence of the whole transgene. No EPSPS fragments were amplifiable in microbial DNA, suggesting that transformation had not occurred during the 48 h incubation. Uptake of transgenic DNA fragments by ruminal bacteria is probably precluded or time-limited by rapid degradation of plant DNA upon plant cell lysis.

http://www.ncbi.nlm.nih.gov/pubmed/15137918

14. Duggan PA, Chambers PA, Heritage J and Forbes JM (2003): Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. British Journal of Nutrition 89: 159-66.

The polymerase chain reaction (PCR) technique was used to investigate the fate of a transgene in the rumen of sheep fed silage and maize grains from an insect-resistant maize line. A 1914-bp DNA fragment containing the entire coding region of the synthetic cryIA(b) gene was still amplifiable from rumen fluid sampled 5 h after feeding maize grains. The same target sequence, however, could not be amplified from rumen fluid sampled from sheep fed silage prepared from the genetically modified maize line. PCR amplification of a shorter (211-bp), yet still highly specific, target sequence was possible with rumen fluid sampled up to 3 and 24 h after feeding silage and maize grains, respectively. These findings indicate that intact transgenes from silage are unlikely to survive significantly in the rumen since a DNA sequence 211-bp long is very unlikely to transmit genetic information. By contrast, DNA in maize grains persists for a significant time and may, therefore, provide a source of transforming DNA in the rumen. In addition, we have examined the biological activity of plasmid DNA that had previously been exposed to the ovine oral cavity. Plasmid extracted from saliva sampled after incubation for 8 min was still capable of transforming competent Escherichia coli to kanamycin resistance, implying that DNA released from the diet within the mouth may retain sufficient biological activity for the transformation of competent oral bacteria.

http://www.ncbi.nlm.nih.gov/pubmed/12575900

15. Koonin EV (2003): Horizontal gene transfer: the path to maturity. Molecular Microbiology 50 (3): 725-27.

The realization that horizontal (lateral) gene transfer (HGT) might have had a major impact on biological evolution is perhaps the most fundamental change in our perception of general aspects of biology brought about by massive genome sequencing (Gogarten et al., 2002; Doolittle et al., 2003). As Lawrence and Hendrickson (2003) rightly point out in the MicroReview appearing in this issue, HGT might also be the most controversial topic in genomics. The main thesis of their review is that the study of HGT is still 'in its adolescence'. They discuss four major questions that, in their reckoning, should be addressed for HGT to graduate to adulthood:

(i) How does HGT impact the evolutionary history of different genes?
(ii) How does the role of HGT differ among different lineages?
(iii) How does one reach robust conclusions on the presence or absence of HGT?
(iv) How does one integrate HGT into the continuum of genetic exchange to arrive at meaningful microbiological concepts?

What is required for HGT studies to progress from youth to maturity? I believe that the key question, once the major evolutionary impact of HGT is considered to have been proven (if it is not, then there is not much point in further discussion), is: why? That is, why are horizontally transferred genes fixed in a microbial population? The general answer is, of course, that fixation is driven by Darwinian selection, i.e. the acquired genes increase the fitness of the recipient (it goes without saying that only a miniscule fraction of foreign DNA that invades a microbial cell is fixed) (Koonin et al., 2001; Berg and Kurland, 2002). However, the big enigma is that horizontally transferred genes can confer advantage on the cells that retain them, although they come from a donor whose evolutionary history is distinct from that of the recipient and should therefore be ill adapted to functioning in the latter. The answer could be relatively obvious in the case of acquisition of 'selfish' operons, which confer new metabolic capacities to the recipient (Lawrence and Roth, 1996; Lawrence and Hendrickson, 2003). In contrast, the selective advantage of xenologous (orthologous) gene displacement, which might be an even more common form of HGT (Koonin et al., 2001), is much harder to contemplate. One clue seems to be provided by antibiotic resistance, e.g. replacement of an antibiotic-sensitive bacterial aminoacyl-tRNA synthetase by a resistant eukaryotic one (Brown et al., 1998). How general this explanation might be, however, remains unclear. Numerous cases of apparent xenologous displacement suggest entirely unexpected compatibility of proteins that are not adjusted to functioning in the same cellular environment. I believe that, when a satisfactory understanding of the underlying causes of fixation of horizontally transferred genes is achieved at both the theoretical and the empirical levels, the study of HGT will reach the status of a mature research area. At that point, the dramatic implications of massive HGT, in particular, for the tree representation of life's history, will have to be faced in earnest.

http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2958.2003.03808.x/full

16. Kay E, Vogel TM, Bertolla F, Nalin R and Simonet P (2002): In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Applied and Environmental Microbiology 68 (7): 3345-3351.

Interkingdom gene transfer is limited by a combination of physical, biological, and genetic barriers. The results of greenhouse experiments involving transplastomic plants (genetically engineered chloroplast genomes) cocolonized by pathogenic and opportunistic soil bacteria demonstrated that these barriers could be eliminated. The Acinetobacter sp. strain BD413, which is outfitted with homologous sequences to chloroplastic genes, coinfected a transplastomic tobacco plant with Ralstonia solanacearum and was transformed by the plant's transgene (aadA) containing resistance to spectinomycin and streptomycin. However, no transformants were observed when the homologous sequences were omitted from the Acinetobacter sp. strain. Detectable gene transfer from these transgenic plants to bacteria were dependent on gene copy number, bacterial competence, and the presence of homologous sequences. Our data suggest that by selecting plant transgene sequences that are nonhomologous to bacterial sequences, plant biotechnologists could restore the genetic barrier to transgene transfer to bacteria.

http://aem.asm.org/content/68/7/3345.Abstract

17. Coghlan A (2002): Does it matter if genes can jump from GM food to bugs in human gut? New Scientist 2353, 27th July 2002, p. 6.

http://www.newscientist.com/article/mg17523530.400-does-it-matter-if-genes-can-jump-from-gm-food-to-bugs-in-human-gut.html

18. Kunik T, Tzfira T, Kapulnik Y, Gafni Y, Dingwall C and Citovsky V (2001): Genetic transformation of HeLa cells by Agrobacterium. Proc Natl Acad Sci USA 98 (4): 1871-6.

Agrobacterium tumefaciens is a soil phytopathogen that elicits neoplastic growths on the host plant species. In nature, however, Agrobacterium also may encounter organisms belonging to other kingdoms such as insects and animals that feed on the infected plants. Can Agrobacterium, then, also infect animal cells? Here, we report that Agrobacterium attaches to and genetically transforms several types of human cells. In stably transformed HeLa cells, the integration event occurred at the right border of the tumor-inducing plasmid's transferred-DNA (T-DNA), suggesting bona fide T-DNA transfer and lending support to the notion that Agrobacterium transforms human cells by a mechanism similar to that which it uses for transformation of plants cells. Collectively, our results suggest that Agrobacterium can transport its T-DNA to human cells and integrate it into their genome.

http://lib.bioinfo.pl/paper:11172043

19. Mercer DK, Scott KP, Melville CM, Glover LA and Flint HJ (2001): Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. FEMS Microbiol Lett. 200 (2): 163-7.

Transformation of Streptococcus gordonii DL1 by free DNA was studied in human saliva. Competent S. gordonii could be transformed in vitro with plasmid DNA that had been taken into the human mouth. Transformation also occurred with a plasmid that cannot replicate in S. gordonii, but that has a region of chromosomal homology, by integration into the bacterial chromosome, although linearised plasmid DNA gave no transformants. Linear chromosomal DNA fragments did however transform S. gordonii/Tn916 efficiently in saliva when regions of homology with the recipient chromosome flanked the marker gene. These findings are discussed in relation to the potential for acquisition of DNA sequences, including genetically modified DNA, by gut and oral bacteria.

http://www.ncbi.nlm.nih.gov/pubmed/11425469

20. Duggan PS, Chambers PA, Heritage J and Forbes JM (2000): Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. FEMS Microbiology Letters 191: 71-77.

To assess the likelihood that the bla gene present in a transgenic maize line may transfer from plant material to the microflora associated with animal feeds, we have examined the survival of free DNA in maize silage effluent, ovine rumen fluid and ovine saliva. Plasmid DNA that had previously been exposed to freshly sampled ovine saliva was capable of transforming competent Escherichia coli cells to ampicillin resistance even after 24 h, implying that DNA released from the diet could provide a source of transforming DNA in the oral cavity of sheep. Although target DNA sequences could be amplified by polymerase chain reaction from plasmid DNA after a 30-min incubation in silage effluent and rumen contents, only short term biological activity, lasting less than 1 min, was observed in these environments, as shown by transformation to antibiotic resistance. These experiments were performed under in vitro conditions; therefore further studies are needed to elucidate the biological significance of free DNA in the rumen and oral cavities of sheep and in silage effluent.

http://www.ncbi.nlm.nih.gov/pubmed/11004402

21. Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA and Flint HJ (1999): Fate of free DNA and transformation of the oral bacterium Streptococcus gordonii DL1 by plasmid DNA in human saliva. Applied and Environmental Microbiology 65 (1): 6-10.

Competitive PCR was used to monitor the survival of a 520-bp DNA target sequence from a recombinant plasmid, pVACMC1, after admixture of the plasmid with freshly sampled human saliva. The fraction of the target remaining amplifiable ranged from 40 to 65% after 10 min of exposure to saliva samples from five subjects and from 6 to 25% after 60 min of exposure. pVACMC1 plasmid DNA that had been exposed to degradation by fresh saliva was capable of transforming naturally competent Streptococcus gordonii DL1 to erythromycin resistance, although transforming activity decreased rapidly, with a half-life of approximately 50 s. S. gordonii DL1 transformants were obtained in the presence of filter-sterilized saliva and a 1-microg/ml final concentration of pVACMC1 DNA. Addition of filter-sterilized saliva instead of heat-inactivated horse serum to S. gordonii DL1 cells induced competence, although with slightly lower efficiency. These findings indicate that DNA released from bacteria or food sources within the mouth has the potential to transform naturally competent oral bacteria. However, further investigations are needed to establish whether transformation of oral bacteria can occur at significant frequencies in vivo.

http://www.ncbi.nlm.nih.gov/pubmed/9872752

22. Gebhard F and Smalla K (1999): Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiology Ecology 28 (3): 261-72.

Field releases of transgenic rizomania-resistant sugar beet (Beta vulgaris) plants were accompanied by a study of the persistence of DNA from transgenic sugar beet litter in soil and of horizontal gene transfer of plant DNA to bacteria. The transgenic sugar beets contained the marker genes nptII and bar under the control of the bidirectional TR1/2 promoter conferring kanamycin (Km) and glufosinate ammonium resistance to the plant. Primer systems targeting the construct allowed the specific and sensitive detection of the transgenic DNA in soil. Soil samples were analyzed by cultivation of bacteria on nonselective and Km-selective media to determine the proportion of Km-resistant bacteria and to monitor the culturable fraction for incorporation of transgenic plant DNA. To detect the presence of transgenic DNA independently from cultivation, total soil DNA was extracted and amplified by PCR with three different primer sets specific for the transgenic DNA. Long-term persistence of transgenic DNA could be shown under field conditions (up to 2 years) and also in soil microcosms with introduced transgenic plant DNA. No construct-specific sequences were detected by dot blot hybridizations of bacterial isolates. The experimental limitations of detecting horizontal gene transfer from plants to bacteria under field conditions are discussed

http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6941.1999.tb00581.x/Abstract

23. Grillot-Courvalin C, Goussand S, Huetz F, Ojcius DM and Courvalin P (1998): Functional gene transfer from intracellular bacteria to mammalian cells. Nature Biotech 16: 862-66.

We provide evidence of direct transfer of functional DNA from bacteria to mammalian cells. An Escherichia coli K12 diaminopimelate auxotroph made invasive by cloning the invasin gene from Yersinia pseudotuberculosis transfers DNA after simple co-incubation, into a variety of mammalian cell lines. Transfer efficiency was enhanced in some cells by coexpression of the gene for listeriolysin from Listeria monocytogenes. Expression of the acquired genes occurs in both dividing and quiescent cells. The only requirement for bacteria to transfer genetic material into nonprofessional phagocytic cells and macrophages is the ability to invade the host cell.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Functional+gene+transfer+from+intracellular+bacteria+to+mammalian+cells.+Nature+Biotech+16%3A+862-66

24. Nielsen KM, Bones AM, Smalla K and van Elsas JD (1998): Horizontal gene transfer from transgenic plants to terrestrial bacteriaa rare event? FEMS Microbiology Reviews 22: 79-103.

Today, 12 years after the first field release of a genetically modified plant (GMP), over 15,000 field trials at different locations have been performed. As new and unique characteristics are frequently introduced into GMPs, risk assessment has to be performed to assess their ecological impact. The possibilities of horizontal gene transfer (HGT; no parent-to-offspring transfer of genes) from plants to microorganisms are frequently evaluated in such risk assessments of GMPs before release into the field. In this review we indicate why putative HGT from plants to terrestrial (soil and plant associated) bacteria has raised concern in biosafety evaluations. Further, we discuss possible pathways of HGT from plants to bacteria, outline the barriers to HGT in bacteria, describe the strategies used to investigate HGT from plants to bacteria and summarize the results obtained. Only a few cases of HGT from eukaryotes such as plants to bacteria have been reported to date. These cases have been ascertained after comparison of DNA sequences between plants and bacteria. Although experimental approaches in both field and laboratory studies have not been able to confirm the occurrence of such HGT to naturally occurring bacteria, recently two studies have shown transfer of marker genes from plants to bacteria based on homologous recombination. The few examples of HGT indicated by DNA sequence comparisons suggest that the frequencies of evolutionarily successful HGT from plants to bacteria may be extremely low. However, this inference is based on a small number of experimental studies and indications found in the literature. Transfer frequencies should not be confounded with the likelihood of environmental implications, since the frequency of HGT is probably only marginally important compared with the selective force acting on the outcome. Attention should therefore be focused on enhancing the understanding of selection processes in natural environments. Only an accurate understanding of these selective events will allow the prediction of possible consequences of novel genes following their introduction into open environments.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Horizontal+gene+transfer+from+transgenic+plants+to+terrestrial+bacteria+%E2%80%93+a+rare+event%3F+FEMS+Microbiology+Reviews+22%3A+79-103

25. Ho M W, Traavik T, Olsvik O, Tappeser B, Howard CV, Weizsacker Cv, McGavin GC (1998): Gene technology and gene ecology of infectious diseases. Microbial Ecology in Health and Disease 10: 33-59.

According to the 1996 WHO Report, the world is heading for a major crisis in public health as outbreaks of new and re-emerging infectious diseases are striking at increasing frequencies within the past 10 to 15 years. The current strains of pathogens are moreover, resistant to known treatments; some strains being resistant to all or nearly all drugs and antibiotics. Horizontal gene transfer is now generally recognized to be responsible for the evolution of virulence and the spread of drug and antibiotic resistances. Many pathogens have crossed species barriers, having acquired genes from phylogenetically distant species that are involved in their ability to cause diseases. Recent findings document the extremely wide scope of horizontal gene transfer and the extensive recombination between genetic material from unrelated species that have contributed to the emergence of virulence and antibiotic resistances. The past 15 years coincide with the development of genetic engineering biotechnology on a commercial scale. Genetic engineering depends on designing vectors for cloning and transferring genes and involves artificially recombining and manipulating genes from unrelated species and their viral pathogens, thereby enhancing the probability for horizontal gene transfer and recombination. The urgent question which needs to be addressed is the extent to which genetic engineering biotechnology, by facilitating horizontal gene transfer and recombination, is contributing to the resurgence of infectious, drug-resistant diseases, and will continue to do so if allowed to proceed unchecked. An enquiry into the possible contribution of genetic engineering biotechnology to the etiology of infectious diseases is all the more pressing in the light of other relevant recent findings indicating that microorganisms genetically engineered for 'contained use' may not be effectively contained. Thus, biologically 'crippled' strains of bacteria can survive in the environment to exchange genes with other species; DNA released from cells is not readily broken down in the environment, thereby retaining the ability to transform organisms; some viral DNA can be more infectious than the virus itself; and routine chemical treatments for inactivating pathogenic microorganisms and viruses, before they are discharged into the environment, may be ineffective, leaving a substantial percentage of pathogens in an active infectious state. The combination of the different kinds of evidence is sufficiently compelling, especially in view of the precautionary principle, to warrant, at the very least, an independent public enquiry into genetic engineering biotechnology and the etiology of infectious diseases.

http://www.isis.org.uk/pdf/gene_technology_and_gene_ecology_infectious_disease.pdf

26. Doerfler W and Schubbert R. (1998): Uptake of foreign DNA from the environment: the gastroinestinal tract and the placenta as portals of entry, Wien Klin Wochenschr. 110, 40-44

Foreign DNA (deoxyribonucleic acid) is part of our environment. Considerable amounts of foreign DNA of very different origin are ingested daily with food. In a series of experiments we fed the DNA of bacteriophage M13 as test DNA to mice and showed that fragments of this DNA survive the passage through the gastrointestinal (GI) tract in small amounts (1-2%). Food-ingested M13 DNA reaches peripheral white blood cells, the spleen and liver via the intestinal epithelia and cells in the Peyer's patches of the intestinal wall. There is evidence to assume that food-ingested foreign DNA can become covalently linked to mouse-like DNA. When M13 DNA is fed to pregnant mice the test DNA can be detected in cells in various organs of the fetuses and of newborn animals, but never in all cells of the mouse fetus. It is likely that the M13 DNA is transferred by the transplacental route and not via the germ line. The consequences of foreign DNA uptake for mutagenesis and oncogenesis have not yet been investigated.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Uptake+of+foreign+DNA+from+the+environment%3A+the+gastroinestinal+tract+and+the+placenta+as+portals+of+entry

27. Schubbert, R., Rentz, D., Schmitzx, B. and Doerfler, W. (1997): Foreign (M13 DNA ingested by mice reaches peripheral leukocytes, spleen and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. Proc. Nat. Acad. Sci. 94, 961-6.

Food-ingested foreign DNA is not completely degraded in the gastrointestinal tract of mice. Phage M13mp18 DNA as a test molecule devoid of homology to mouse DNA was pipette-fed to or added to the food supply of mice. The fate of this foreign DNA in the animals was followed by several methods. In 84 animals, fragments of M13mp18 DNA were detected in the contents of the small intestine, the cecum (until 18 h), the large intestine, or the feces. In 254 animals, M13mp18 DNA fragments of up to 976 bp were found in blood 2-8 h after feeding. In buffer-fed control animals, M13mp18 DNA could not be detected. M13mp18 DNA fragments were traced by PCR in peripheral leukocytes and located by fluorescent in situ hybridization in about 1 of 1000 white cells between 2 and 8 h, and in spleen or liver cells up to 24 h after feeding, but not later. M13mp18 DNA could be traced by fluorescent in situ hybridization in the columnar epithelial cells, in the leukocytes in Peyer's patches of the cecum wall, in liver cells, and in B cells, T cells, and macrophages from spleen. These findings suggest transport of foreign DNA through the intestinal wall and Peyer's patches to peripheral blood leukocytes and into several organs. Upon extended feeding, M13mp18 DNA could be recloned from total spleen DNA into a lambda vector. Among about 2.5 x 10(7) lambda plaques, one plaque was isolated that contained a 1299 nucleotide pair fragment (nt 4736-6034) of sequence-identified M13mp18 DNA. This fragment was covalently linked to an 80 nt DNA segment with 70% homology to the mouse IgE receptor gene. The DNA from another lambda plaque also contained mouse DNA, bacterial DNA, and rearranged lambda DNA. Two additional plaques contained M13mp18 DNA fragments of at least 641 (nt 2660-3300) or 794 (nt 4640-5433) nucleotide pairs. The medical and evolutionary implications of these observations may be considerable.

http://www.ncbi.nlm.nih.gov/pubmed/?term=Foreign+%28M13+DNA+ingested+by+mice+reaches+peripheral+leukocytes%2C+spleen+and+liver+via+the+intestinal+wall+mucosa+and+can+be+covalently+linked+to+mouse+DNA

GENE FLOW, CONTAMINATION & FIELD TRIALS' RISK

1. **Nicola Schoenenberger and Luigi D'Andrea (2012): Surveying the occurrence of subspontaneous glyphosate-tolerant genetically engineered Brassica napus L. (Brassicaceae) along Swiss railways. Environmental Sciences Europe 2012, 24:23**

Railway tracks represent a highly interlinked habitat with numerous possibilities for accidental entry of oilseed rape due to seed spill during transportation. Moreover, glyphosate is regularly employed to control the vegetation, increasing the possibility of establishment for plants resistant to it. We surveyed the presence of genetically engineered glyphosate tolerant oilseed rape (Brassica napus) with a focus on the most important Swiss railway stations. Our objective was to detect accidental establishment of transgenic plants, since Switzerland does not import nor cultivate transgenic oilseed rape. Seventy-nine railway areas were sampled in Switzerland and the Principality of Liechtenstein, and the feral presence of oilseed rape was detected in 58 of them. A total of 2403 individuals were tested for genetic modification using commercially available immunologic test kits. In four localities, one located in Lugano and three in the area of Basel, a total of 50 plants expressing the CP4 EPSPS protein were detected. In two of the localities, survival of herbicide applications was observed. The populations were probably introduced through contaminated seed spills from freight trains, or during the transfer of goods from cargo ships to trains. Railways represent an ideal system for herbicide resistant transgenic plants to establish and spread as a result of high selective pressure in favour of herbicide resistance with consequent increased difficulties to keep the infrastructure free of weeds. Crop-to-wild gene flow can occur as several sexually compatible species which are congeneric or in allied genera to oilseed rape were found growing sympatrically. Moreover, the capillary presence of railways in the agricultural landscape provides a putative source of contamination of GE-free agriculture. Our results suggests that carefully adapted monitoring designs may be set in order

to detect introduction events that can lead to rapid establishment and growing populations as the accepted contamination thresholds are likely to be biologically insufficient to prevent further environmental contamination. The complete article is available as a provisional PDF.

http://www.enveurope.com/content/pdf/2190-4715-24-23.pdf

2. **Munier DJ, Brittan KL, Lanini WT. (2012): Seed bank persistence of genetically modified canola in California. Environ Sci Pollut Res Int. 19(6):2281-4.**

Canola which is genetically modified (GM) for tolerance to glyphosate has the potential to become established as a new glyphosate resistant weed, thus reducing the effectiveness of glyphosate. Volunteers from dormant canola seeds produced thousands of plants per acre in the fourth year (2011) following a 2007 crop harvest. This occurred with no additional canola seed production since the 2007 harvest. Volunteer plants following harvests of annual crops are typically only a problem for the first year after harvest. In California, glyphosate is the core herbicide on millions of acres of high value row, tree, and vine crops and new glyphosate resistant weeds reduce the effectiveness of glyphosate. The combination of dormant seed and herbicide resistance makes GM glyphosate resistant canola a new and difficult California weed which was first observed in the winter of 2009.

http://wric.ucdavis.edu/PDFs/Seed_bank_persistence_of_genetically_modified_canola.pdf

3. Heuberger S, Ellers-Kirk C, Tabashnik BE, Carrière Y (2010): Pollen- and Seed-Mediated Transgene Flow in Commercial Cotton Seed Production Fields. PLoS ONE 5(11): e14128

Characterizing the spatial patterns of gene flow from transgenic crops is challenging, making it difficult to design containment strategies for markets that regulate the adventitious presence of transgenes. Insecticidal Bacillus thuringiensis (Bt) cotton is planted on millions of hectares annually and is a potential source of transgene flow. Here we monitored 15 non-Bt cotton (Gossypium hirsutum, L.) seed production fields (some transgenic for herbicide resistance, some not) for gene flow of the Bt cotton cry1Ac transgene. We investigated seed-mediated gene flow, which yields adventitious Bt cotton plants, and pollen-mediated gene flow, which generates outcrossed seeds. A spatially-explicit statistical analysis was used to quantify the effects of nearby Bt and non-Bt cotton fields at various spatial scales, along with the effects of pollinator abundance and adventitious Bt plants in fields, on pollen-mediated gene flow. Adventitious Bt cotton plants, resulting from seed bags and planting error, comprised over 15% of plants sampled from the edges of three seed production fields. In contrast, pollen-mediated gene flow affected less than 1% of the seed sampled from field edges. Variation in outcrossing was better explained by the area of Bt cotton fields within 750 m of the seed production fields than by the area of Bt cotton within larger or smaller spatial scales. Variation in outcrossing was also positively associated with the abundance of honey bees. A comparison of statistical methods showed that our spatially-explicit analysis was more powerful for understanding the effects of surrounding fields than customary models based on distance. Given the low rates of pollen-mediated gene flow observed in this study, we conclude that careful planting and screening of seeds could be more important than field spacing for limiting gene flow.

4. Galeano, Pablo, Debat, Claudio Martínez, Ruibal, Fabiana, Fraguas, Laura Franco and Galván, Guillermo A. (2010): Cross-fertilization between genetically modified and non-genetically modified maize crops in Uruguay. Env Biosafety Res Vol 9 (3): 147-154

The cultivation of genetically modified (GM) Bt maize (Zea mays L.) events MON810 and Bt11 is permitted in Uruguay. Local regulations specify that 10% of the crop should be a non-GM cultivar as refuge area for biodiversity, and the distance from other non-GM maize crops should be more than 250 m in order to avoid cross-pollination. However, the degree of cross-fertilization between maize crops in Uruguay is unknown. The level of adventitious presence of GM material in non-GM crops is a relevant issue for organic farming, in situ conservation of genetic resources and seed production. In the research reported here, the occurrence and frequency of cross-fertilization between commercial GM and non-GM maize crops in Uruguay was assessed. The methodology comprised field sampling and detection using DAS-ELISA and PCR. Five field-pair cases where GM maize crops were grown near non-GM maize crops were identified. These cases had the potential to cross-fertilize considering the distance between crops and the similarity of the sowing dates. Adventitious presence of GM material in the offspring of non-GM crops was found in three of the five cases. Adventitious presence of event MON810 or Bt11 in non-GM maize, which were distinguished using specific primers, matched the events in the putative sources of transgenic pollen. Percentages of transgenic seedlings in the offspring of the non-GM crops were estimated as 0.56%, 0.83% and 0.13% for three sampling sites with distances of respectively 40, 100 and 330 m from the GM crops. This is a first indication that adventitious presence of transgenes in non-GM maize crops will occur in Uruguay if isolation by distance and/or time is not provided. These findings contribute to the evaluation of the applicability of the "regulated coexistence policy" in Uruguay.

5. Song X, Liu L, Wang Z and Qiang S (2009): Potential gene flow from transgenic rice (Oryza sativa L.) to different weedy rice (Oryza sativa f. spontanea) accessions based on reproductive compatibility. Pest Management Science. Vol 65 (8): 862-9

The possibility of gene flow from transgenic crops to wild relatives may be affected by reproductive capacity between them. The potential gene flow from two transgenic rice lines containing the bar gene to five accessions of weedy rice (WR1WR5) was determined through examination of reproductive compatibility under controlled pollination. The pollen grain germination of two transgenic rice lines on the stigma of all weedy rice, rice pollen tube growth down the style and entry into the weedy rice ovary were similar to self-pollination in weedy rice. However, delayed double fertilisation and embryo abortion in crosses between WR2 and Y0003 were observed. Seed sets between transgenic rice lines and weedy rice varied from 8 to 76%. Although repeated pollination increased seed set significantly, the rank of the seed set between the weedy rice accessions and rice lines was not changed. The germination rates of F1 hybrids were similar or greater compared with respective females. All F1 plants expressed glufosinate resistance in the presence of glufosinate selection pressure. The frequency of gene flow between different weedy rice accessions and transgenic herbicide-resistant rice may differ owing to different reproductive compatibility. This result suggests that, when wild relatives are selected as experimental materials for assessing the gene flow of transgenic rice, it is necessary to address the compatibility between transgenic rice and wild relatives.

http://onlinelibrary.wiley.com/doi/10.1002/ps.1766/Abstract

6. Snow A (2009): Unwanted transgenes re-discovered in Oaxacan maize. Molecular Ecology 18: 569-571.

In summary, this study confirms what many people have long suspected that transgenes have entered landrace maize populations in Oaxaca. Based on the scant published literature and the fact that Pineyro-Nelson et al. found some transgenic plants in three communities, we do not have enough information to estimate the frequencies of transgenic plants in this region or others, or to know whether introductions are still taking place. Mexican farmers are known to plant seeds of modern varieties of maize alongside their local landraces (Perales et al. 2003), thereby allowing genes from imported grain to introgress into landraces, perhaps irreversibly. Apparently, DICONSA no longer distributes GM maize, but Mexico still imports yellow GM maize from the USA for food and animal feed. Looking towards the future, containment of commercial maize transgenes will be impossible and eradication from landraces will be difficult (CEC 2004), but preventative measures and confinement of introduced transgenes are viable goals (Bitocchi et al. 2008). Further surveys will be helpful for identifying centres of introduction and preserving essentially GM-free zones of maize landraces in Mexico. Meanwhile, the uneven acceptance of GM crops around the globe will continue to spawn disputes over the sovereignty of national gene pools.

http://www.biosci.ohio-state.edu/~asnowlab/Snow_MolEcol_Transgenesin%20Maixe.pdf

7. Pineyro-Nelson A, Van Heerwaarden J, Perales HR, Serratos-Hernandez JA, Rangel A et al (2009): Transgenes in Mexican maize Molecular evidence and methodological considerations for GMO detection in landrace populations. Mol Ecology 18: 750-61.

A possible consequence of planting genetically modified organisms (GMOs) in centres of crop origin is unintended gene flow into traditional landraces. In 2001, a study reported the presence of the transgenic 35S promoter in maize landraces sampled in 2000 from the Sierra Juarez of Oaxaca, Mexico. Analysis of a large sample taken from the same region in 2003 and 2004 could not confirm the existence of transgenes, thereby casting doubt on the earlier results. These two studies were based on different sampling and analytical procedures and are thus hard to compare. Here, we present new molecular data for this region that confirm the presence of transgenes in three of 23 localities sampled in 2001. Transgene sequences were not detected in samples taken in 2002 from nine localities, while directed samples taken in 2004 from two of the positive 2001 localities were again found to contain transgenic sequences. These findings suggest the persistence or re-introduction of transgenes up until 2004 in this area. We address variability in recombinant sequence detection by analyzing the consistency of current molecular assays. We also present theoretical results on the limitations of estimating the probability of transgene detection in samples taken from landraces. The inclusion of a limited number of female gametes and, more importantly, aggregated transgene distributions may significantly lower detection probabilities. Our analytical and sampling considerations help explain discrepancies among different detection efforts, including the one presented here, and provide considerations for the establishment of monitoring protocols to detect the presence of transgenes among structured populations of landraces.

http://www.biomedsearch.com/nih/Transgenes-in-Mexican-maize-molecular/19143938.html

8. D'Hertefeldt T, Jørgensen RB, Pettersson LB. (2008): Long term persistence of GM oilseed rape in the seed bank. Biol Lett. 4 (3): 314-7

Coexistence between genetically modified (GM) and non-GM plants is a field of rapid development and considerable controversy. In crops, it is increasingly important to understand and predict the GM volunteer emergence in subsequent non-GM crops. Theoretical models suggest recruitment from the seedbank over extended periods, but empirical evidence matching these predictions has been scarce. Here, we provide evidence of long-term GM seed persistence in conventional agriculture. Ten years after a trial of GM herbicide-tolerant oilseed rape, emergent seedlings were collected and tested for herbicide tolerance. Seedlings that survived the glufosinate herbicide (15 out of 38 volunteers) tested positive for at least one GM insert. The resulting density was equivalent to 0.01 plants m-2, despite complying with volunteer reduction recommendations. These results are important in relation to debating and regulating coexistence of GM and non-GM crops, particularly for planting non-GM crops after GM crops in the same field.

http://www.ncbi.nlm.nih.gov/pubmed/18381261

9. Knispel A.L., McLachlan, S.M., Van Acker, R., Friesen, L.F. (2008): Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science 56, 7280

Genetically modified herbicide-tolerant (GMHT) oilseed rape (OSR; Brassica napus L.) was approved for commercial cultivation in Canada in 1995 and currently represents over 95% of the OSR grown in western Canada. After a decade of widespread cultivation, GMHT volunteers represent an increasing management problem in cultivated fields and are ubiquitous in adjacent ruderal habitats, where they contribute to the spread of transgenes. However, few studies have considered escaped GMHT OSR populations in North America, and even fewer have been conducted at large spatial scales (i.e. landscape scales). In particular, the contribution of landscape structure and large-scale anthropogenic dispersal processes to the persistence and spread of escaped GMHT OSR remains poorly understood. We conducted a multi-year survey of the landscape-scale distribution of escaped OSR plants adjacent to roads and cultivated fields. Our objective was to examine the long-term dynamics of escaped OSR at large spatial scales and to assess the relative importance of landscape and localised factors to the persistence and spread of these plants outside of cultivation.

http://www.environmentalconservationlab.ca/wp-content/uploads/2010/06/alexis1.pdf

10. Warwick S I, Legere A, Simard M J and James T (2008): Do escaped transgenes persist in nature? The case of a herbicide resistance transgene in a weedy Brassicarapa population. Molecular Ecol. Vol. 17 (5): 1387-95.

The existence of transgenic hybrids resulting from transgene escape from genetically modified (GM) crops to wild or weedy relatives is well documented but the fate of the transgene over time in recipient wild species populations is still relatively unknown. This is the first report of the persistence and apparent introgression, i.e. stable incorporation of genes from one differentiated gene pool into another, of an herbicide resistance transgene from Brassica napus into the gene pool of its weedy relative, Brassica rapa, monitored under natural commercial field conditions. Hybridization between glyphosate-resistant [herbicide resistance (HR)]B. napus and B. rapa was first observed at two Québec sites, Ste Agathe and St Henri, in 2001. B. rapa populations at these two locations were monitored in 2002, 2003 and 2005 for the presence of hybrids and transgene persistence. Hybrid numbers decreased over the 3-year period, from 85 out of approximately 200 plants surveyed in 2002 to only five out of 200 plants in 2005 (St Henri site). Most hybrids had the HR trait, reduced male fertility, intermediate genome structure, and presence of both species-specific amplified fragment length polymorphism markers. Both F(1) and backcross hybrid generations were detected. One introgressed individual, i.e. with the HR trait and diploid ploidy level of B. rapa, was observed in 2005. The latter had reduced pollen viability but produced approximately 480 seeds. Forty-eight of the 50 progeny grown from this plant were diploid with high pollen viability and 22 had the transgene (1:1 segregation). These observations confirm the persistence of the HR trait over time. Persistence occurred over a 6-year period, in the absence of herbicide selection pressure (with the exception of possible exposure to glyphosate in 2002), and in spite of the fitness cost associated with hybridization.

http://www.curehunter.com/public/pubmed17971090.do

11. Heinemann, J.A. (2007): A typology of the effects of (trans)gene flow on the conservation and sustainable use of genetic resources. Commission on Genetic Resources for Food and Agriculture.

Managing transgene flow is considered by some to be the same challenge as managing the flow of any exotic or other gene that is considered to be a threat to human health, the environment or IP. In terms of the physical and biological strategies for managing gene flow, this view is probably correct. In terms of the types of harms that might result from the failure of management, however, this view is not generally agreed. In part, it cannot be true for some genes, such as those that produce potent human pharmaceutical agents, dsRNA with the ability to silence human genes, or allergens the genes for which would not be present in the twelve plants that supply 95% of crop based foods for humans (Adi, 2006). Such genes may never have been part of their genomes or if they had been, would long ago have been eliminated by human selection against such plants.

Uncertainties in gene flow remain concentrated in these areas:

- what will be the harms or benefits that transgene flow may create?
- can management reduce the frequency or impact of any potential harms that may result from transgene flow to acceptable levels for the most dangerous commercialized or field tested transgenes?
- what are the cumulative effects of transgene flow on species, crops, conservation areas, and soci-cultural frameworks?
- what are the consequences of transgene flow when considered in the context of liability laws, international trade and market expectations, intellectual property (IP) rights and differentiated market certification programs

Conclusion: Future research priorities should focus on key scientific uncertainties about the impacts of transgene flow. These include identifying the characteristics of transgenes that may contribute to their introgression via fitness-enhancing effects and in introgression pathways that do not depend on the immediate selective value of the transgene to plants. This will require a combination of evolutionary and population genetics and researchers with expertise in global genetic change. It is worth stressing the point that introgression is not necessary for some hypothetical harms to derive from transgene flow. Even local, single flow events could cause harm. For example, the escape into another crop of a transgene that makes a human allergen, or the escape of a transgene that makes seeds sterile into an endangered wild relative. Therefore, the focus should remain on the potential for transgene flow for some transgenes, not just the effects of introgression. Complex and unanticipated effects of gene stacking should be explored. Most importantly, the potentially destructive effects of transgene flow from PMPs and PMIPs on human health and biodiversity require discussion of policies or invention of containment options that will prevent transgene flow. Future policy priorities should focus on creating a more uniform and constructive approach to distributing the benefits of biotechnology without imposing penalties on those farmers and consumers who chose not to use the products of biotechnology. In particular, changes should be encouraged where necessary to legal systems that place responsibility for containing GM crops on those who sell them and their products.

http://ir.canterbury.ac.nz/bitstream/10092/3236/1/12605322_bsp35r1e.pdf

12. Zelaya I A, Owen M D K, VanGessel M J (2007): Transfer of glyphosate-resistance: Evidence of hybridisation in Conyza (Asteraceae). Amer. J. Botany 94: 660-673

Transfer of herbicide resistance genes between crops and weeds is relatively well documented; however, far less information exists for weed-to-weed interactions. The hybridization between the weedy diploids Conyza canadensis(2n = 18) and C. ramosissima (2n = 18) was investigated by monitoring transmission of the allele conferring resistance to N-phosphonomethyl glycine (glyphosate). In a multivariate quantitative trait analysis, we described the phylogenic relationship of the plants, whereas we tested seed viability to assess potential postzygotic reproductive barriers (PZRB) thus affecting the potential establishment of hybrid populations in the wild. When inflorescences were allowed to interact freely, approximately 3% of C. ramosissima or C. canadensis ova were fertilized by pollen of the opposing species and produced viable seeds; >95% of the ova were fertilized under no-pollen competition conditions (emasculation). The interspecific Conyza hybrid demonstrated an intermediate phenotype between the parents but superior resistance to glyphosate compared to the resistant C. canadensis parent. Inheritance of glyphosate resistance in the selfed ( ) followed the partially dominant nuclear, single-gene model; backcrosses confirmed successful introgression of the resistance allele to either parent. Negligible PZRB were observed in the hybrid progenies, confirming fertility of the C. canadensis × C. ramosissima nothotaxa. The implications of introgressive hybridization for herbicide resistance management and taxonomy of Conyza are discussed.

http://www.amjbot.org/content/94/4/660.full

13. Serratos-Hernández J-A, Gómez-Olivares J-L, Salinas-Arreortua N, Buendía-Rodríguez E, Islas-Gutiérrez F and de-Ita A (2007): Transgenic proteins in maize in the Soil Conservation area of Federal District, Mexico. Frontiers in Ecology and the Environment 5: 247-252.

In 2003, the environmental authorities of the Federal District of Mexico declared that genetically modified organisms were incompatible with ecological agriculture practices established in rural areas south of Mexico City. To ensure compliance with official standards and organic agriculture policies, steps were taken to implement an early warning system for the detection of genetically modified maize in farmers' fields. In our sampling efforts, which were conducted in 2003, transgenic proteins expressed in maize were found in two (0.96%) of 208 samples from farmers' fields, located in two (8%) of 25 sampled communities. Mexico imports a substantial amount of maize from the US, and due to formal and informal seed networks among rural farmers, there are many potential routes of entrance for transgenic maize into food and feed webs. To sustain agroecological practices, preserve organic agriculture, and conserve maize landraces in the Soil Conservation area of the Mexican Federal District, environmental authorities will need to maintain and update ecological policies such as the "green seal" for organic agriculture, apply alternative technologies such as biofertilizers to enhance plant nutrition, and develop sustainable maize agriculture with the implementation of profitable intercropping systems.

http://www.jstor.org/discover/10.2307/20440649?uid=3737496&uid=2&uid=4&sid=21101680663671

14. Simard M J, Legere A and Warwick SI (2006): Transgenic Brassica napus fields and Brassica rapa weeds in Quebec sympatry and weed-crop in situ hybridization. Canadian Journal of Botany-Revue Canadienne De Botanique 84: 1842-1851.

Hybridization between the herbicide-resistant transgenic crop Brassica napus L. (canola) and its weedy relative Brassica rapa L. (bird rape) has been documented in Quebec. Our goal was to evaluate the actual hybridization potential based on range overlap and actual in situ hybridization rates. This was done by mapping B. napus canola fields, comparing them with the sampling locations of B. rapa herbarium specimens from Quebec, gathering information on the presence of B. rapa in certified canola seed production fields, and surveying for B. rapa populations located in, or close to B. napus field margins. Progeny from these populations were screened for herbicide resistance (HR) and for the presence of the HR transgene. Two fields were also selected to evaluate B. rapa density effects on hybridization rates. Significant sympatry was observed in several areas of the province; hybridization occurred in all eight populations (1.1% to 17.5% hybrid seed) located in field margins and in one (1.1%) out of three populations located less than 10 m from a B. napus field. Hybridization rates decreased exponentially as B. rapa density increased, but interplant rates (0% to 68%) were highly variable. Environmental problems could be generated by the release of B. napus crops with traits conferring fitness benefits in nonmanaged areas.

15. Légère A (2005): Riks and consequences of gene flow from herbicide-resistant crops: canola (Brassica napus L) as a case study. Pest Manag. Sci. 61 (3): 292-300.

Data from the literature and recent experiments with herbicide-resistant (HR) canola (Brassica napus L) repeatedly confirm that genes and transgenes will flow and hybrids will form if certain conditions are met. These include sympatry with a compatible relative (weedy, wild or crop), synchrony of flowering, successful fertilization and viable offspring. The chance of these events occurring is real; however, it is generally low and varies with species and circumstances. Plants of the same species (non-transgenic or with a different HR transgene) in neighbouring fields may inherit the new HR gene, potentially generating plants with single and multiple HR. For canola, seed losses at harvest and secondary dormancy ensures the persistence over time of the HR trait(s) in the seed bank, and the potential presence of crop volunteers in subsequent crops. Although canola has many wild/weedy relatives, the risk of gene flow is quite low for most of these species, except with Brassica rapa L. Introgression of genes and transgenes in B rapa populations occurs with apparently little or no fitness costs. Consequences of HR canola gene flow for the agro-ecosystem include contamination of seed lots, potentially more complex and costly control strategy, and limitations in cropping system design. Consequences for non-agricultural habitats may be minor but appear largely undocumented.

http://www.ncbi.nlm.nih.gov/pubmed/15593291

16. Watrud LS, Lee EH, Fairbrother A, Burdick C, Reichman JR, Bollman M, Storm M, King G and Van de Water PK (2004): Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. PNAS Vol. 101 (40). 14533-14538.

Sampling methods and results of a gene flow study are described that will be of interest to plant scientists, evolutionary biologists, ecologists, and stakeholders assessing the environmental safety of transgenic crops. This study documents gene flow on a landscape level from creeping bentgrass (Agrostis stolonifera L.), one of the first wind-pollinated, perennial, and highly outcrossing transgenic crops being developed for commercial use. Most of the gene flow occurred within 2 km in the direction of prevailing winds. The maximal gene flow distances observed were 21 km and 14 km in sentinel and resident plants, respectively, that were located in primarily nonagronomic habitats. The selectable marker used in these studies was the CP4 EPSPS gene derived from Agrobacterium spp. strain CP4 that encodes 5-enol-pyruvylshikimate-3-phosphate synthase and confers resistance to glyphosate herbicide. Evidence for gene flow to 75 of 138 sentinel plants of A. stolonifera and to 29 of 69 resident Agrostis plants was based on seedling progeny survival after spraying with glyphosate in greenhouse assays and positive TraitChek, PCR, and sequencing results. Additional studies are needed to determine whether introgression will occur and whether it will affect the ecological fitness of progeny or the structure of plant communities in which transgenic progeny may become established.

http://www.ncbi.nlm.nih.gov/pubmed/15448206

17. Vacher C, Weis AE, Hermann D, Kossler T, Young C and Hochbert ME (2004): Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (Brassica rapa). Theor Appl Genet 109 (4): 806-14.

The inevitable escape of transgenic pollen from cultivated fields will lead to the emergence of transgenic crop-wild plant hybrids in natural patches of wild plants. The fate of these hybrids and that of the transgene depend on their ability to compete with their wild relatives. Here we study ecological factors that may enhance the fitness of genetically modified hybrids relative to wild plants for a Bacillus thuringiensis (Bt) transgene conferring resistance to insects. Mixed stands of wild plants and first-generation hybrids were grown under different conditions of herbivore pressure and density, with Bt oilseed rape (Brassica napus) as the crop and B. rapa as the wild recipient. Biomass and fitness components were measured from plant germination to the germination of their offspring. The frequency of transgenic seedlings in the offspring generation was estimated using the green fluorescent protein marker. The biomass of F(1) Bt-transgenic hybrids relative to that of wild-type plants was found to be sensitive to both plant density and herbivore pressure, but herbivore pressure appeared as the major factor enhancing their relative fitnesses. In the absence of herbivore pressure, Bt hybrids produced 6.2-fold fewer seeds than their wild neighbors, and Bt plant frequency fell from 50% to 16% within a single generation. Under high herbivore pressure, Bt hybrids produced 1.4-fold more seeds, and Bt plant frequency was 42% in the offspring generation. We conclude that high-density patches of highly damaged wild plants are the most vulnerable to Bt-transgene invasion. They should be monitored early to detect potential transgene spread.

http://www.ncbi.nlm.nih.gov/pubmed/15340690

18. Messeguer J., Marfa V., Catala M.M., Guiderdoni E., Melé E.. (2004): A field study of pollen-mediated gene flow from Mediterranean GM rice to conventional rice and the red rice weed. Molecular Breeding 13 (1), 103- 112.

The objective of this study was to assess the frequency of pollen-mediated gene flow from a transgenic rice line, harbouring the gusA and the bar genes encoding respectively, [bêta]-glucuronidase and phosphinothricin acetyl transferase as markers, to the red rice weed and conventional rice in the Spanish japonica cultivar Senia. A circular field trial design was set up to investigate the influence of the wind on the frequency of pollination of red rice and conventional rice recipient plants with the transgenic pollen. Frequencies of gene flow based on detection of herbicide resistant, GUS positive seedlings among seed progenies of recipient plants averaged over all wind directions were 0.036 ± 0.006% and 0.086 ± 0.007 for red rice and conventional rice, respectively. However, for both red rice and conventional rice, a clear asymmetric distribution was observed with pollination frequency favoured in plants placed under the local prevailing winds. Southern analyses confirmed the hemizygous status and the origin of the transgenes in progenies of surviving, GUS positive plants. Gene flow detected in conventional rice planted at 1, 2, 5 and 10 m distance revealed a clear decrease with increasing distance which was less dramatic under the prevailing wind direction. Consequences of these findings for containment of gene flow from transgenic rice crops to the red rice weed are discussed. The precise determination of the local wind conditions at flowering time and pollination day time appear to be of primary importance for setting up suitable isolation distances.

19. Mellon M and Rissler J (2004): Gone to Seed: Transgenic Contaminants in the Traditional Seed Supply, Union of Concerned Scientists

Most of the transgenes used by genetic engineers are new to foods and some are not intended for use in foods at all. For these and other reasons, concerns have arisen about the possibility that transgenes introduced into crop varieties through genetic engineering might unintentionally contaminate the seed supply for traditional, or non-genetically engineered, varieties of crops. The research covered in this report addresses (that) possibility with a small pilot study of seeds of traditional varieties of three major food crops: corn, soybeans and canola. The study found that the seeds of traditional varieties bought from the same retailers used by US farmers are pervasively contaminated with low levels of DNA sequences originating in genetically engineered varieties of those crops. This conclusion is based on tests conducted by two respected commercial laboratories using duplicate samples of seeds of six traditional varieties each of corn, soybeans, and canola. One laboratory detected trasngenically derived in DNA in 50 percent of the corn, 50 percent of the soybean and 100 percent of the traditional canola varieties tested. The other laboratory detected transgenically derived DNA in 83% of the traditional varieties of each of the three crops.

20. Ramsay G, Thompson C and Squire G (2004): Quantifying landscape-scale gene flow in oil seed rape, Scottish Crop Research Institute and U.K Department of Environment, Food and Rural Affairs, (DEFRA), October 2004, p.4

6. Gene flow was detected over long distances. To facilitate a mathematical description of the decline in fertilisation and to characterise better the long tail in the distribution, sites with male-sterile plants were set out at 5 and 26 km from the nearest known source with little expectation of finding pollination. Low levels of fertilisation occurred at both sites. Although the origin of such events is hard to ascribe with absolute certainty, it appears that they were due to normal natural pollination via vectors which operate over long distances.
(THIS IS NOT THE FULL EXECUTIVE SUMMARY)

21. Poppy GM (2004): Gene flow from GM plants: towards a more quantitative risk assessment. Trends in Biotechnology 22 (9): 436-8.

Assessing the risks associated with geneflow from GM crops to wild relatives is a significant scientific challenge. Most researchers have focused on assessing the frequency of gene flow, too often on a localized scale, and ignoring the hazards caused by geneflow. To quantify risk, multi-disciplinary research teams need to unite and scale up their studies.

http://www.ncbi.nlm.nih.gov/pubmed/15331221

22. Stewart Jr. CN, Halfhill MD and Warwick SI (2003): Transgene introgression from genetically modified crops to their wild relatives. Nature Reviews Genetics 4: 806-817.

Transgenes engineered into annual crops could be unintentionally introduced into the genomes of their free-living wild relatives. The fear is that these transgenes might persist in the environment and have negative ecological consequences. Are some crops or transgenic traits of more concern than others? Are there natural genetic barriers to minimize gene escape? Can the genetic transformation process be exploited to produce new barriers to gene flow? Questions abound, but luckily so do answers.

http://www.nature.com/nrg/journal/v4/n10/abs/nrg1179.html#top

23. Snow AA (2002): Transgenic crops: why gene flow matters. Nature Biotechnology Vol. 20 (6): 542.

Examines the ecological and agronomic effects of gene flow from transgenic crops. Introduction of genes into ecosystems; Impact of crop gene introgression into plant population; Prevention of gene flow between sexually-compatible species.

http://connection.ebscohost.com/c/articles/8782446/transgenic-crops-why-gene-flow-matters

24. Gealy DR, Mitten DH and Rutger JN (2003): Gene flow between red rice (Oryza sativa) and herbicide-resistant rice (O. sativa)-Implications for weed management. Weed Technology Vol. 17: 627-645.

Red rice is a major weed of rice in the southern U.S. and can intercross with rice. Knowledge of the plant phenotypes from such crosses would be valuable for identification and management of these plants. Male-sterile long-grain tropicaljaponicas 'Kaybonnet-1789' and 'Cypress-1819' were crossed with two awned and two awnless U.S. red rice types. F1 plants produced pubescent leaves, red pericarp, and medium-grain seeds. Crosses involving awned LA3 and TX4 red rice produced F1 plants with reddish-purple basal leaf sheaths and usually flowered within the same time periods as the parents, whereas those involving awnless StgS red rice had green basal leaf sheaths, flowered much later than either parent, and produced awnless F1 and F2 offspring. Crosses involving awned red rice produced F1 plants with long awns and F2 plants with awns ranging in length from zero to that of red rice parents. F1 plants were taller than either parent and produced intermediate culm angles similar to red rice, whereas F2 plants had culms ranging from erect (like rice) to more open than red rice. Thus, true F1hybrids from crosses between pure breeding (homozygous) rice and red rice can be positively identified by a combination of traits including pubescent leaves, medium-grain seeds with red pericarps, open plant types, and heights greater than the red rice parent. F1 hybrids may be awned or awnless, have purple or green stems, or have normal or delayed heading. F2 plants have a broad combination of phenotypic traits found in both parents and F1 hybrids

http://wssajournals.org/doi/abs/10.1614/WT-05-066.1?journalCode=wete

25. Friesen L F., Nelson, A G & Van Acker, R C., (2003): Evidence of contamination of pedigreed canola (brassica napus) seed lots in western Canada with genetically engineered herbicide resistance traits. Agronomy Journal, 95 (5), 1342 1347

The objective of this study was to survey pedigreed canola (Brassica napus L.) seedlots for contaminating herbicide resistance traits because of complaints from farmers regarding glyphosate [N-(phosphonomethyl)glycine]-resistant canola volunteers occurring unexpectedly in their fields at densities and in patterns that suggested that pollen-mediated gene flow from neighboring fields in previous years was not the source of contamination. Twenty-seven unique, commercial certified canola seedlot samples were collected. Glyphosate-resistant seedlot samples were not collected. Canola samples were planted in the field, and when the canola had two to four true leaves, glyphosate, glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid], and thifensulfuron {methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate} herbicides were applied. Surviving canola plants were counted. Of the 27 seedlots, 14 had contamination levels above 0.25% and therefore failed the 99.75% cultivar purity guideline for certified canola seed. Three seedlots had glyphosate resistance contamination levels in excess of 2.0%. Unexpected contamination (even at 0.25%) can cause problems for producers that practice direct seeding and depend on glyphosate for nonselective, broad-spectrum weed control. To avoid unexpected problems and costs, it is important that farmers are cognizant of the high probability that pedigreed canola seedlots are cross-contaminated with the various herbicide resistance traits.

26. Rieger MA, Lamond M, Preston C, Powles SB, Roush RT (2002): Pollen-mediated movement of herbicide resistance between commercial canola fields. Science 296 (5577): 2386-2388.

There is considerable public and scientific debate for and against genetically modified (GM) crops. One of the first GM crops, Brassica napus (oilseed rape or canola) is now widely grown in North America, with proposed commercial release into Australia and Europe. Among concerns of opponents to these crops are claims that pollen movement will cause unacceptable levels of gene flow from GM to non-GM crops or to related weedy species, resulting in genetic pollution of the environment. Therefore, quantifying pollen-mediated gene flow is vital for assessing the environmental impact of GM crops. This study quantifies at a landscape level the gene flow that occurs from herbicide-resistant canola crops to nearby crops not containing herbicide resistance genes.

http://www.sciencemag.org/content/296/5577/2386.Abstract

27. Eastham, K. & Sweet, J. (2002): Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen.

This report considers the significance of pollen-mediated gene flow from six major crop types that have been genetically modified and are close to commercial release in the European Union. Oilseed rape, sugar beet, potatoes, maize, wheat and barley are reviewed in detail using recent and current research findings to assess their potential environmental and agronomic impacts. There is also a short review on the current status of GM fruit crops in Europe. Each crop type considered has its own distinctive characteristics of pollen production, dispersal and potential outcrossing, giving varying levels of gene flow.

Oilseed rape can be described as a high-risk crop for crop-to-crop gene flow and from crop to wild relatives. At the farm scale low levels of gene flow will occur at long distances and thus complete genetic isolation will be difficult to maintain. This particularly applies to varieties and lines containing male sterile components, which will outcross with neighbouring fully fertile GM oilseed rape at higher frequencies and at greater distances than traditional varieties. Gene stacking in B. napus has been observed in crops and it is predicted that plants carrying multiple resistance genes will become common post-GM release and consequently GM volunteers may require different herbicide management. Oilseed rape is crosscompatible with a number of wild relatives and thus the likelihood of gene flow to these species is high.

Sugar beet can be described as medium to high risk for gene flow from crop to crop and from crop to wild relatives. Pollen from sugar beet has been recorded at distances of more than 1 km at relatively high frequencies. Cross-pollination in root crops is not usually considered an issue since the crop is harvested before flowering. However a small proportion of plants in a crop will bolt and transgene movement between crops may occur in this way. Hybridisation and introgression between cultivated beet and wild sea beet has been shown to occur.

Potatoes can be described as a low risk crop for gene flow from crop to crop and from crop to wild relatives. Cross-pollination between production crops is not usually considered an issue since the harvested tuber is not affected by incoming pollen. In true seed production areas, however, the likelihood of cross-pollination between adjacent crops

leading to contamination is higher. The risk of gene flow exists if volunteers are allowed to persist in a field from one crop to the next. Naturally occurring hybridisation and introgression between potato and its related wild species in Europe is unlikely.

Maize can be described as a medium to high-risk crop for gene flow from crop to crop. Evidence suggests that GM maize plants would cross-pollinate non-GM maize plants up to and beyond their recommended isolation distance of 200 m. There are no known wild relatives in Europe with which maize can hybridise.

Wheat can be described as a low risk crop for gene flow from crop to crop and from crop to wild relatives. Cross-pollination under field conditions normally involves less than 2 % of all florets so any outcrossing usually occurs with adjacent plants. Hybrids formed between wheat and several wild barley and grass species generally appear to be restricted to the first generation with little evidence for subsequent introgression due to sterility.

Barley can be described as a low risk crop for gene flow from crop to crop and from crop to wild relatives. Barley reproduces almost entirely by self-fertilisation, producing small amounts of pollen so that most outcrossing occurs between closely adjacent plants. There are no records of naturally occurring hybrids between barley and any wild relatives in Europe.

Some fruit crops, such as strawberry, apple, grapevine and plum have outcrossing and hybridisation tendencies which suggest that gene flow from GM crops to other crops and to wild relatives is likely to occur. For raspberry, blackberry and blackcurrant the likelihood of gene flow is less easy to predict, partly due to lack of available information.

At present none of these crops has pollen which can be completely contained. This means that the movement of seed and pollen will have to be measured and managed much more in the future. Management systems such as spatial and temporal isolation can be used to minimise direct gene flow between crops, and to minimise seed bank and volunteer populations. The use of isolation zones, crop barrier rows and other vegetation barriers between pollen source and recipient crops can reduce pollen dispersal, although changing weather and environmental conditions mean that some long distance pollen dispersal will occur. Biological containment measures are being developed that require research in order to determine whether plant reproduction can be controlled to inhibit gene flow through pollen and/or seed.

The possible implications of hybridisation and introgression between crops and wild plant species are so far unclear because it is difficult to predict how the genetically engineered genes will be expressed in a related wild species. The fitness of wild plant species containing introgressed genes from a GM crop will depend on many factors involving both the genes introgressed and the recipient ecosystem. While it is important to determine frequencies of hybridisation between crops and wild relatives, it is more important to determine whether genes will be introgressed into wild populations and establish at levels which will have a significant ecological impact.

http://www.eea.eu.int/

28. Wheeler CC, Gealy D and TeBeest DO (2001): Bar gene transfer from transgenic rice (Oryza sativa) to red rice (Oryza sativa). Rice Research: AAES Res. Series 485: 33-37

There are many new developments in biotechnology, and among them are transgenic glufosinate-tolerant rice cultivars, which appear to provide a solution to the question of controlling red rice in cultivated rice. However, outcrossing between red rice and cultivated rice has been documented since 1961, and this is an issue that should be addressed first. Field and greenhouse experiments were conducted to determine if glufosinate-tolerant genes were transferred to red rice by outcrossing with transgenic white rice and at what rate the outcrossing occurred. Preliminary review of the data gathered from experiments in which seedlings were treated with glufosinate suggests that red rice seedlings resistant to glufosinate are produced in significant numbers as a result of out-crossing; however, molecular evidence of gene transfer is needed to confirm gene transfer to red rice.

http://arkansasagnews.uark.edu/485.pdf

29. Hall L, Topinka K, Huffman J, Davis L and Good A (2000): Pollen flow between herbicide-resistant Brassica napus is the cause of multiple-resistant B-napus volunteers. Weed Science 48: 688-694

A field in which Brassica napus volunteers were not controlled by several applications of glyphosate was investigated in 1998. This field had been planted with glufosinate-resistant and imidazolinone-resistant B. napus in 1997 and was adjacent to a field that had grown glyphosate-resistant B. napus. Mature volunteer B. napus were collected on a 50- by 100-m grid in the field. Progeny from 34 volunteers were sprayed with glyphosate at 440 g ae ha1, and the survivors were sprayed with either glufosinate or imazethapyr at 400 or 50 g ai ha1, respectively. Where seed numbers permitted (14 volunteers), seedlings were also sprayed sequentially with glyphosate, glufosinate, and imazethapyr, at 440 g ae ha1, 400 g ai ha1, and 50 g ai ha1, respectively. In total, 15 volunteers had progeny that were between 66 and 82% resistant to glyphosate, consistent with the predicted 3:1 resistant:susceptible ratio. Volunteer B. napus plants with glyphosate-resistant seedlings were most common close to the putative pollen source; however, a plant with glyphosate-resistant progeny was collected 500 m from the adjacent field edge. Seedlings from all nine volunteers collected from the glufosinate-resistant area showed multiple resistance to glyphosate and glufosinate, whereas seedlings from 10 of 20 volunteers collected from the imidazolinone-resistant area showed resistance to imazethapyr and glyphosate. DNA extraction and restriction fragment length polymorphism (RFLP) analysis of seedlings confirmed that mature B. napus volunteers were hybrids resulting from pollen transfer rather than inadvertent seed movement between fields. Two seedlings from the 924 screened were resistant to all three herbicides. Progeny from these self-pollinated individuals were resistant to glyphosate and glufosinate at the predicted 3:1 resistant:susceptible ratio and resistant to imazethapyr at the predicted 15:1 resistant:susceptible ratio. Sequential crossing of three herbicide-resistant varieties is the most likely explanation for the observed multiple herbicide resistance. Integrated management techniques, including suitable crop and herbicide rotations, herbicide mixtures, and nonchemical controls should be used to reduce the incidence and negative effect of B. napus volunteers with multiple herbicide resistance.

http://www.jstor.org/discover/10.2307/4046338?uid=3738256&uid=2&uid=4&sid=21101649517353

30. Fisk, M.C., Whittington, J. 2010. Bayer loses fifth straight trial over US rice crops. Bloomberg Businessweek, July 14.

Bayer AG lost its fifth straight trial over contaminated U.S. long-grain rice to a Louisiana farmer who claimed the company's carelessness with its genetically engineered seed caused exports to plunge. A jury in St. Louis said today the company should pay damages of $500,248. The company previously lost two trials in state court and two in federal, for a total of more than $52 million in jury awards. It faces about 500 additional lawsuits in federal and state courts with claims by 6,600 plaintiffs. It hasn't won any rice trials so far.

http://www.businessweek.com/news/2010-07-14/bayer-loses-fifth-straight-trial-over-u-s-rice-crops.html

31. Dawson A. (2009): CDC Triffid flax scare threatens access to no. 1 EU market. Manitoba Co-operator, September 17. Dawson A. (2009): Changes likely for flax industry. Manitoba Cooperator, September 24.

Like a movie monster that refuses to die, CDC Triffid, a genetically modified (GM) Canadian flax deregistered in 2001, has surfaced in Germany, European Union (EU) officials believe. Like a movie monster that refuses to die, CDC Triffid, a genetically modified (GM) Canadian flax deregistered in 2001, has surfaced in Germany, European Union (EU) officials believe.

And flax prices have plummeted just as farmers feared they might when they lobbied to have the variety voluntarily pulled from the market. Although the Canadian Food Inspection Agency (CFIA) declared CDC Triffid safe, the EU has not yet approved GM flax. Earlier this summer, the EU found the genetic marker NPTII in two cargoes of Canadian flax, indicating it had been genetically modified.

Barry Hall, president of the Flax Council of Canada says it's hard to fathom how CDC Triffid, which was never commercialized, could be showing up now. As of last week, Hall said he hasn't seen any laboratory results proving the flax in question is CDC Triffid. But a reliable source said signs are pointing in that direction.

http://www.agcanada.com/manitobacooperator/2009/09/17/cdc-triffid-flax-scare-threatens-access-to-no-1-eu-market/

32. Blue E.N. 2007. Risky business. Economic and regulatory impacts from the unintended release of genetically engineered rice varieties into the rice merchandising system of the US. Greenpeace International.

http://www.greenpeace.org/raw/content/international/press/reports/riskybusiness.pdf

33. Schmitz T.G., Schmitz A., Moss C.B. (2005): The economic impact of StarLink corn. Agribusiness 21, 391407.

The discovery of StarLink corn in U.S. food products caused considerable disruption in corn markets in 2000 and 2001. Segregation costs were incurred by the U.S. grain-handling system in order to ensure that domestic and export sales of food corn and export sales of non-food corn to Japan meet stringent tolerance levels. These costs reduced the revenue that U.S. corn producers would have received in the absence of StarLink. However, the Loan Deficiency Payment Program (LDP) effectively reduced the loss in revenue attributed to StarLink. This study develops a partial equilibrium model that encompasses both segregation costs and the LDP program in order to obtain empirical estimates of the impact of StarLink on U.S. corn producers over the 2000/2001 marketing year. It is estimated that StarLink caused U.S. producers to lose between $26 and $288 million in revenue.

http://onlinelibrary.wiley.com/doi/10.1002/agr.20054/Abstract

YIELD MYTHS WITH GM CROPS

1. Doug Gurian-Sherman (2012): High and Dry-Why Genetic Engineering Is Not Solving Agriculture's Drought Problem in a Thirsty World. Union of Concerned Scientists.

The Union of Concerned Scientists (UCS) analyzed the prospects for improving crops in ways that can reduce water use overall, and losses during dry periods. We focused on crop genetic engineeringthe lab-based manipulation of genes from any source to alter plants. Practitioners and proponents have touted the potential of genetic engineering to address drought. Biotech companies, including Monsanto, have promised to deliver new crop varieties engineered with novel genes that enable them to thrive under drought conditions. The biotech industry has also suggested that genetic engineering can reduce demand for water from crops even under normal conditionsresulting in "more crop per drop." However, we found little evidence of progress in making crops more water efficient. We also found that the overall prospects for genetic engineering to significantly address agriculture's drought and water-use challenges are modest at best.

Monsanto's gene will confer only modest protection against moderate droughtabout 6 percent more than non-engineered varieties used in Monsanto's test plots five or six years ago. This outcome, based on only two years of field trials with widely varying results, may not accurately predict the level of drought tolerance once the product is grown more widely.

By comparison, classical breeding techniques and improved farming practices have increased drought tolerance in U.S. corn by an estimated 1 percent per year over the past several decades, according to one recent study (due to the challenges of measuring drought tolerance, this value should be considered a rough estimate).

That means traditional methods of improving drought tolerance may have been two to three times as effective as genetic engineering, considering the 10 to 15 years typically required to produce a genetically

engineered crop. If traditional approaches have improved corn's drought tolerance by just 0.3 percent to 0.4 percent per year, they have provided as much extra drought protection as Monsanto's GE corn over the period required to develop it.

(Extracts from the Executive Summary)

2. Zobiole LHS, Kremer RJ, Oliveira RS, Constantin J. (2011): Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans Journal of Applied Microbiology. 110: 118-127

Glyphosate-resistant (GR) soybean production increases each year because of the efficacy of glyphosate for weed management. A new or 'second' generation of GR soybean (GR2) is now commercially available for farmers that is being promoted as higher yielding relative to the previous, 'first generation' (GR1) cultivars. Recent reports show that glyphosate affects the biology and ecology of rhizosphere micro-organisms in GR soybean that affect yield. The objective of this research was to evaluate the microbiological interactions in the rhizospheres of GR2 and GR1 soybean and the performance of the cultivars with different rates of glyphosate applied at different growth stages. A greenhouse study was conducted using GR1 and GR2 soybean cultivars grown in a silt loam soil. Glyphosate was applied at V2, V4 and V6 growth stages at three rates. Plants harvested at R1 growth stage had high root colonization by Fusarium spp.; reduced rhizosphere fluorescent pseudomonads, Mn-reducing bacteria, and indoleacetic acidproducing rhizobacteria; and reduced shoot and root biomass. Glyphosate applied to GR soybean, regardless of cultivar, negatively impacts the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity. The information presented here will be crucial in developing strategies to overcome the potential detrimental effects of glyphosate in GR cropping systems.

3. Zobiole, L H S, Kremer, R J, Oliveira, R S & Constantin, J. (2011): Glyphosate affects chlorophyll, nodulation and nutrient accumulation of "second generation" glyphosate-resistant soybean (Glycine max L.) Pesticide Biochemistry and Physiology 99: 53-60

The recently developed "second generation" of Roundup ReadyÒ soybean (RR2) cultivars commercially available for farmers in 2008 were promoted as higher yielding relative to the "first generation" RR cultivars (RR1). Previous studies showed that glyphosate reduced such yield components as photosynthesis, water absorption, nutrient uptake and symbiotic N2 fixation in RR soybean cultivars; however, no data are available regarding the glyphosate effects on these physiological factors in RR2 soybean. Thus, the objective of this research was to evaluate the nutrient accumulation and nodulation of both generations of RR soybeans at different rates of glyphosate applied at various growth stages. In general, increased glyphosate rates and late applications decreased the nutrient accumulation, nodulation, and shoot and root biomass in both RR1 and RR2. All macro- and micronutrients, with exception of N and K, accumulated more in RR1 than RR2. Although this result may be an individual cultivar characteristic, it suggests that the RR2 cultivar was also inefficient in nutrient uptake and translocation or was unable to rapidly recover from potential chelating effects of glyphosate. These studies suggest that applying glyphosate at early growth stages using the lowest glyphosate rate might have less damage on growth and productivity of RR soybeans.

4. L.H.S. Zobiole, R.S. Oliveira Jr, R.J. Kremer, J. Constantin, T. Yamada, C. Castro, F.A. Oliveira, A. Oliveira Jr (2010): Effect of glyphosate on symbiotic N2 fixation and nickel concentration in glyphosate-resistant soybeans. Applied Soil Ecology 44: 176180

Decreased biological nitrogen fixation in glyphosate-resistant (GR) soybeans has been attributed directly to toxicity of glyphosate or its metabolites, to N2-fixing microorganisms. As a strong metal chelator, glyphosate could influence symbiotic N2 fixation by lowering the concentration of nickel (Ni) that is essential for the symbiotic microorganisms. Evaluation of different cultivars grown on different soil types at the State University of Maringa, PR, Brazil during the summer 2008 revealed, significant decreases in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) and nickel content with glyphosate use (single or sequential application). This work demonstrated that glyphosate can influence the symbiotic N2 fixation by lowering nickel content available to the symbiotic microorganisms.

5. Gurian-Sherman, D. (2009): Failure to yield - Evaluating the performance of genetically engineered crops. Union of Concerned Scientists

The burgeoning human population challenges agriculture to come up with new tools to increase crop productivity. At the same time, we must not simply produce more food at the expense of clean air, water, soil, and a stable climate, which future generations will also require. In order to invest wisely in the future, we must evaluate agricultural tools to see which ones hold the most promise for increasing intrinsic and operational yields and pro¬viding other resource benefits.It is also important to keep in mind where increased food production is most neededin developing countries, especially in Africa, rather than in the developed world. Several recent studies have shown that low-external-input methods such as organic can improve yield by over 100 percent in these countries, along with other benefits. Such methods have the advantage of being based largely on knowledge rather than on costly inputs, and as a result they are often more accessible to poor farmers than the more expensive technologies (which often have not helped in the past). So far, the record of GE crops in contributing to increased yield is modest, despite considerable effort. There are no transgenic crops with increased intrin¬sic yield, and only Bt corn exhibits somewhat higher operational yield. Herbicide-tolerant soybeans, the most widely utilized GE crop by far, do not increase either operational or intrinsic yield. Genetic engineers are working on new genes that may raise both intrinsic and operational yield in the future, but their past track record for bring¬ing new traits to market suggests caution in relying too heavily on their success. It is time to look more seriously at the other tools in the agricultural toolkit. While GE has received most of the attention and investment, tra¬ditional breeding has been delivering the goods in the all-important arena of increasing intrinsic yield. Newer and sophisticated breeding methods using increasing genomic knowledgebut not GEalso show promise for increasing yield. The large investment in the private sector ensures that research on GE versions of major crops will continue, while organic and other agro-ecological methods are not likely to attract a similar investment. But given the modest yield increases from transgenic crops so far, putting too many of our crop-development eggs in the GE basket could lead to lost opportunities. Thus it is very impor¬tant to compare the potential contributions of GE with those of other approaches, such as organic methods, low-input methods, and enhanced conventional-breeding methods. Where these alter¬natives look more promising, we should provide sufficient public funding to ensure that they will be available. Such prioritization is especially appro¬priate for research aimed at developing countries, where yield increases are most needed.

http://www.ucsusa.org/assets/documents/food_and_agriculture/failure-toyield.pdf

6. Kuruganti K (2009): Bt cotton and the myth of enhanced yields. Economic & Political Weekly, Vol. 44 (22)

It is presumed that remarkable increases in cotton productivity in India have come about through bacillus thuringiensis cotton and that this approach therefore must be replicated in other crops. This article explores the myth of rising yields of genetically modified crops and points out that genetic engineering has been at best neutral with respect to yield and in many cotton growing countries the average cotton yields have stagnated since the adoption of Bt cotton.

7. FARSUL. (2009): Divulgados resultados do Programa de Avaliação de Cultivares de Soja (Published results of the Program Evaluation of soybean cultivars). 17/06/2009.

In 2009, Brazilian farmer organization FARSUL published the results of trials on 61 varieties of soybean (40 GM and 21 non-GM), showing that the average yield of non-GM soybeans was 9 per cent higher than GM, at a cost equivalent production.

http://www.farsul.org.br/pg_informes.php?id_noticia=870

8. Gordon B. (2006): Manganese nutrition of glyphosate resistant and conventional soybeans. Better Crops 91, April.

This study was conducted to determine if glyphosate-resistant (GR) soybeans respond differently to Mn fertilizer than conventional soybean varieties in an irrigated high-yield environment, and if so to develop fertilization strategies that will prevent or correct deficiencies. Yield of the GR variety was less than the conventional variety without Mn fertilizer. However, Mn application (banded at planting) to the GR variety closed the yield gap. The conventional soybean variety was not responsive to Mn fertilization. Conversely, yield was reduced at the highest rate of Mn. A second phase of the study showed that a combination of Mn applied as starter and foliar application provided maximum yield response.

9. S. Eker, L. Ozturk, A. Yazici, B. Erenoglu, V. Romheld, I. Cakmak I (2006): Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (Helianthus annuus L.) plants, J. Agric. Food Chem. 54 (26). PP 1001910025.

Evidence clearly shows that cationic micronutrients in spray solutions reduce the herbicidal effectiveness of glyphosate for weed control due to the formation of metal-glyphosate complexes. The formation of these glyphosate-metal complexes in plant tissue may also impair micronutrient nutrition of nontarget plants when exposed to glyphosate drift or glyphosate residues in soil. In the present study, the effects of simulated glyphosate drift on plant growth and uptake, translocation, and accumulation (tissue concentration) of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were investigated in sunflower (Helianthus annuus L.) plants grown in nutrient solution under controlled environmental conditions. Glyphosate was sprayed on plant shoots at different rates between 1.25 and 6.0% of the recommended dosage (i.e., 0.39 and 1.89 mM glyphosate isopropylamine salt). Glyphosate applications significantly decreased root and shoot dry matter production and chlorophyll concentrations of young leaves and shoot tips. The basal parts of the youngest leaves and shoot tips were severely chlorotic. These effects became apparent within 48 h after the glyphosate spray. Glyphosate also caused substantial decreases in leaf concentration of Fe and Mn while the concentration of Zn and Cu was less affected. In short-term uptake experiments with radiolabeled Fe (59Fe), Mn (54Mn), and Zn (65Zn), root uptake of 59Fe and 54Mn was significantly reduced in 12 and 24 h after application of 6% of the recommended dosage of glyphosate, respectively. Glyphosate resulted in almost complete inhibition of root-to-shoot translocation of 59Fe within 12 h and 54Mn within 24 h after application. These results suggest that glyphosate residues or drift may result in severe impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.

10. Huber D.M., Cheng M.W. and Winsor B.A. (2005): Association of severe Corynespora root rot of soybean with glyphosate-killed giant ragweed. Phytopathology 95, S45.

The soilborne pathogen Corynespora cassiicola was the predominant fungus isolated from severely stunted soybeans adjacent to glyphosate-killed giant ragweed plants (Ambrosia trifida) in Indiana fields. Soybeans adjacent to glyphosate-killed ragweed exhibited dark-brown to black lesions on 9095% of their roots and hypocotyls. In contrast, soybeans that were not adjacent to dead Ambrosia trifida, or that were adjacent to living ragweed plants, exhibited only 510% root rot; and a number of different soilborne fungi in addition to Corynespora were isolated from these roots. Dead ragweed roots generally yielded pure cultures of Rhizoctonia and were not colonized by Corynespora. Koch's postulates were completed in the greenhouse where typical hypocotyl lesions developed in 35 days and lateral, "fine feeder roots" were extensively rotted by Corynespora. Soybean yield reduction was related to the density of glyphosate-killed ragweed plants and ranged from 1.5 kg per dead ragweed to 6 kg per dead ragweed in replicated field plots with and without killed ragweed plants. These field observations indicate that glyphosate or metabolites in dying ragweed root exudates modify the soil environment to predispose adjacent glyphosate-resistant soybean roots to severe Corynespora root rot even at temperatures above 20°C.

11. US Department of Agriculture (2002): The adoption of bioengineered crops.

http://www.ers.usda.gov/publications/aer810/aer810.pdf

Use of crop biotechnology products, such as genetically engineered (GE) crops with input traits for pest management, has risen dramatically since commercial approval in the mid-1990s. This report addresses several of the economic dimensions regarding farmer adoption of bioengineered crops, including herbicidetolerant and insect-resistant varieties. In particular, the report examines: (1) the extent of adoption of

bioengineered crops, their diffusion path, and expected adoption rates over the next few years; (2) factors affecting the adoption of bioengineered crops; and (3) farm-level impacts of the adoption of bioengineered crops. Data used in the analysis are mostly from USDA surveys.

12. Elmore R.W., Roeth, F.W., Nelson, L.A., Shapiro, C.A., Klein, R.N., Knezevic, S.Z., Martin, A. (2001): Glyphosate-resistant soyabean cultivar yields compared with sister lines. Agronomy Journal 93, 408412.

Herbicide-resistant crops like glyphosate resistant (GR) soybean [Glycine max (L.) Merr.] are gaining acceptance in U.S. cropping systems. Comparisons from cultivar performance trials suggest a yield suppression may exist with GR soybean. Yield suppressions may result either cultivar genetic differentials, the GR gene/gene insertion process, or glyphosate. Grain yield of GR is probably not affected by glyphosate. Yield suppression due to the GR gene or its insertion process (GR effect) has not been reported. We conducted a field experiment at four Nebraska locations in 2 yr to evaluate the GR effect on soybean yield. Five backcross-derived pairs of GR and non- GR soybean sister lines were compared along with three high-yield, nonherbicide-resistant cultivars and five other herbicide-resistant cul- tivars. Glyphosate resistant sister lines yielded 5% (200 kg ha21) than the non-GR sisters (GR effect). Seed weight of the non-GR sisters was greater than that of the GR sisters (in 1999) and the non-GR sister lines were 20 mm shorter than the GR sisters. Other variables monitored were similar between the two cultivar groups. The high-yield, nonherbicide-resistant cultivars included for comparison yielded 5% more than the non-GR sisters and 10% more than the GR sisters.

13. Benbrook C. (1999): Evidence of the magnitude and consequences of the Roundup Ready soybean yield drag from university-based varietal trials in 1998. Ag BioTech InfoNet Technical Paper No 1, Jul 13.

This report reviews the results of over 8,200 university-based soybean varietal trials in 1998 and reaches the following conclusions regarding the magnitude of the RR soybean yield drag

* The yield drag between top RR varieties compared to top conventional varieties averages 4.6 bushels per acre, or 6.7 percent.
* When comparing average yields across the top 5 varieties tested in 8 states, the yield drag averages 4.1 bushels, or 6.1 percent.
* Across all varieties tested, the yield drag averages 3.1 bushels, or 5.3 percent.
* In some areas of the Midwest, the best conventional variety sold by seed companies produces yields on average 10 percent or more higher than comparable Roundup Ready varieties sold by the same seed companies.

It is important to place the RR soybean yield drag in perspective. From 1975 to 1994 soybean yields rose on average about 0.5 bushels per year. In 1999 the RR soybean yield drag could result in perhaps a 2.0 to 2.5 percent reduction in national average soybean yields, compared to what they would likely have been if seed companies had not dramatically shifted breeding priorities to focus on herbicide tolerance. If not reversed by future breeding enhancements, this downward shift in soybean yield potential could emerge as the most significant decline in a major crop ever associated with a single genetic modification. On whether RR soybean systems reduce pesticide use and increase grower profits, our analysis shows that RR soybean systems are largely dependent on herbicides and hence are not likely to reduce herbicide use or reliance. Claims otherwise are based on incomplete information or analytically flawed comparisons that do not tell the whole story. Farmers growing RR soybeans used 2 to 5 times more herbicide measured in pounds applied per acre, compared to the other popular weed management systems used on most soybean fields not planted to RR varieties in 1998. RR herbicide use exceeds the level on many farms using multitactic Integrated Weed Management systems by a factor of 10 or more. There is clear evidence that Roundup use by farmers planting RR soybeans has risen markedly in 1999 because of the emergence of a degree of tolerance to Roundup in several key weed species, shifts in weeds toward those less sensitive to Roundup, price cuts and aggressive marketing. Roundup use on soybeans may well double from 1998 levels within the next few years. But if current trends continue in the way RR technology is used, the efficacy and market share of Roundup may then fall just as quickly. The RR soybean yield drag and technology fee impose a sizable indirect tax on the income of soybean producers, ranging from a few percent where RR varieties work best to over 12 percent of gross income per acre. The remarkable popularity of Roundup Ready soybeans, despite their cost and the significant yield drag associated with their use, is evidence of the difficulty and high cost of today's herbicide-dependent soybean weed management systems. The rapid evolution of weeds better able to withstand applications of Roundup reinforces the need for more integrated, multiple tactic weed management systems.

OTHER RELATED PAPERS
(Regulation, Ethics, Corporate Monopolies, Social impacts etc.)

1. **Patrick van Zwanenberg and Valeria Arza (2013): Biotechnology and its configurations: GM cotton production on large and small farms in Argentina. Technology in Society. In Press. Available online Feb. 15th 2013**

Drawing on a socio-technical systems perspective we compare the ways in which novel genetically modified (GM) crop artefacts, related devices and techniques, actors, practices, and institutions have been linked together, or configured, across two distinctive cotton production systems in north east Argentina, one based around large-scale farming and the other based around small-scale family farming. In the former system, new GM seeds, actors, complementary artefacts, agricultural techniques, and technical support, and modified supply markets and regulatory rules have been linked together in ways that mean agricultural biotechnologies perform well. In the latter system, the new GM artefacts were unavailable, whilst conventional seeds disappeared from input markets. Instead, linkages were formed between informal seed multipliers and dealers, copied GM seeds, of unreliable identify and poor quality, unmodified production practices, declining technical support, uncontrolled pest problems, and an absence of regulatory oversight, resulting in a poorly performing technology. In effect, working agricultural biotechnologies are different in the two farming systems; they have different characteristics and capabilities and perform in different ways.

http://dx.doi.org/10.1016/j.techsoc.2013.01.007

New Report Exposes Devastating Impact of Monsanto Practices on U.S. Farmers
Center for Food Safety and Save Our Seeds, February 12 2013

http://www.centerforfoodsafety.org/wp-content/uploads/2013/02/ Seed-Giants_final.pdf

2. **Jonathan Latham and Allison Wilson (2013): Regulators Discover a Hidden Viral Gene in Commercial GMO Crops. Independent Science News.**

Synopsis: A scientific paper published in late 2012 shows that US and EU GMO regulators have for many years been inadvertently approving transgenic events containing an unsuspected viral gene. As a result, 54 different transgenic events commercialized internationally contain a substantial segment of the multifunctional Gene VI from Cauliflower Mosaic Virus (CaMV) within them. Among these are some of the most widely grown GMOs, including Roundup Ready Soybean (40-3-2) and MON810 Maize. The oversight occurred because regulators failed to appreciate that Gene VI overlaps the commonly used CaMV 35S gene regulatory sequence. The authors of the paper, working for the European Food Safety Authority, concluded that functions of Gene VI were potential sources of harmful consequences. They further concluded that, if expressed, the fragments of Gene VI are substantial enough for them to be functional (Podevin and du Jardin (2012) GM Crops and Food 3: 1-5). This discovery has multiple ramifications for biotechnology. Foremost, there is the immediate question of GMO safety and whether the 54 events should be recalled, but secondly, the failure implicates regulators and the industry in a circle of mutual incompetence and complacency. The discovery will also strengthen the argument for GMO labeling: if regulators and industry cannnot protect the public then why should they not be allowed to protect themselves?

http://independentsciencenews.org/commentaries/regulators-discover-a-hidden-viral-gene-in-commercial-gmo-crops/

3. Jack A Heinemann, Sarah Zanon Agapito-Tenfen and Judy A Carman (May 2013): A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environment International. May 2013 Vol. 55: 43-55

Changing the nature, kind and quantity of particular regulatory-RNA molecules through genetic engineering can create biosafety risks. While some genetically modified organisms (GMOs) are intended to produce new regulatory-RNA molecules, these may also arise in other GMOs not intended to express them. To characterise, assess and then mitigate the potential adverse effects arising from changes to RNA requires changing current approaches to food or environmental risk assessments of GMOs. We document risk assessment advice offered to government regulators in Australia, New Zealand and Brazil during official risk evaluations of GM plants for use as human food or for release into the environment (whether for field trials or commercial release), how the regulator considered those risks, and what that experience teaches us about the GMO risk assessment framework. We also suggest improvements to the process.

4. Binimelis, R., Hilbeck, A., Lebrecht T., Vogel R., Heinemann J. (2012): Farmer's choice of seeds in five regions under different levels of seed market concentration and GM crop adoption, GMLS Conference 2012

http://www.gmls.eu/

5. Public Research, Private Gain: Corporate Influence Over University Agricultural Research Food and Water Watch, April 26 2012

The report, Public Research, Private Gain: Corporate Influence Over University Agricultural Research, provides a history of the land-grant university system including how, as public funding has stalled in recent decades, these universities have turned to agribusiness to fill the void, compromising the public mission of the institutions.

http://www.foodandwaterwatch.org/pressreleases/public-research-private-gain-corporate-influence-over-university-agricultural-research/

6. William Sanjour. Designed to Fail: Why Regulatory Agencies Don't Work. Independent Science News, May 1 2012

http://independentsciencenews.org/health/designed-to-fail-why-regulatory-agencies-dont-work/

7. Hilbeck A, Meier M, Trtikova M. (2012): Underlying reasons of the controversy over adverse effects of Bt toxins on lady beetle and lacewing larvae. Environmental Sciences Europe. 24:9

We outline important underlying reasons that fuel the decades-long controversy over adverse effects of Bt toxins expressed in genetically modified plants on beneficial, nontarget organisms. Inconsistent evaluation standards and asymmetrical levels of scrutiny applied to studies reporting significant adverse effects compared to those finding no adverse effects are described using the examples of the green lacewing (Chrysoperla carnea) and the two-spotted lady beetle (Adalia bipunctata). Additionally, the chosen style and concerted nature of the rather confrontational counter study and responses in the lady beetle cases bear striking similarities to other reported examples in the field of biosafety/risk science of genetically modified plants and to other fields of applied industrial techno-science that suggest deeper issues that go well beyond science. We call for a constructive and respectful scientific discourse where moving the frontiers of our collective knowledge forward takes center stage. Reported phenomena based on robust data must not be rejected or delegitimized on their being surprising and lacking an explained mechanism at the time of their discovery. Exploring mechanisms often requires entirely different expertise and methodologies than those of the discoverers. In particular, in biosafety/risk sciences, plurality of arguments and critical research approaches have to be embraced and actively encouraged rather than discredited or even silenced if we are to learn our 'late lessons' from past technology introductions.

8. Glenn Davis Stone (2012): Constructing Facts- Bt Cotton Narratives in India. Economic & Political Weekly, Sept. 22, 2012 Vol. XLVII. No. 38.

A group of researchers and industry writers have constructed a narrative of technological triumph for Bt cotton in India, based on an empirical record of superior performance compared to conventional seed. Counterclaims of Bt cotton failure are attributed to mutually reinforcing interactions among non-governmental organisations which avoid rigorous comparisons. However, researchers and the biotechnology industry are also engaged in a similar authentication loop for generating, validating, and publicising such facts. With Bt cotton, the convention of routinely ignoring the effects of selection bias and cultivation bias benefits researchers, journals and the industry, but keeps us from drawing meaningful conclusions about the relative performance of the technology. But as poor as the case for isolating the technology impact of Bt cotton in India has been, it is useful in helping us understand the social conventions for creating one's "own facts".

9. Glenn Davis Stone and Dominic Glover (2011): Genetically modified crops and the 'food criris': discourse and material impacts. Development in Practice. 21:4-5, 509-516

A surge of media reports and rhetorical claims depicted genetically modified (GM) crops as a solution to the 'global food crisis' manifested in the sudden spike in world food prices during 2007-08. Broad claims were made about the potential of GM technologies to tackle the crisis, even though the useful crops and traits typically invoked had yet to be developed, and despite the fact that real progress had in fact been made by using conventional breeding. The case vividly illustrates the instrumental use of food-crisis rhetoric to promote GM crops.

10. András Székács, Gabriele Weiss, David Quist, Eszter Takács, Béla Darvas, Matthias Meier, Trilochan Swain & Angelika Hilbeck (2012): Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay, Food and Agricultural Immunology, Vol. 23 (2)

A laboratory ring trial was performed in four laboratories for determination of Cry1Ab toxin in leaf material of MON 810 maize using a standardised enzyme-linked immunoassay protocol. Statistical analysis was carried out by the ISO 5725-2 guidelines, sample standard deviation and standard error, within-laboratory and inter-laboratory SD and SE were calculated. Measured inter-laboratory average values were 12.5±4.0, 15.3±4.6 and 72.6±17.8 µg/g for three lyophilised samples, and 27.8±4.3 µg/g for a frozen sample, yet, Cry1Ab concentrations ranged 66.5160.1% of the corresponding IA. Determined concentrations by in-house protocols were statistically not different in one laboratory and different in two laboratories from the corresponding values by the joint protocol. Results emphasise the importance of a standardised protocol among laboratories for comparable quantitative Cry1Ab toxin determination. However, even when using a standardised protocol, significant differences still occur among toxin concentrations detected in different laboratories, although with a smaller range of variation.

11. Michael E. Gray. (2011): "Relevance of Traditional Integrated Pest Management (IPM) Strategies for Commercial Corn Producers in a Transgenic Agroecosystem: A Bygone Era?" Journal of Agricultural and Food Chemistry. 59 (11), pg. 58525858.

The use of transgenic Bt maize hybrids continues to increase significantly across the Corn Belt of the United States. In 2009, 59% of all maize planted in Illinois was characterized as a “stacked” gene variety. This is a 40% increase since 2006. Stacked hybrids typically express one Cry protein for corn rootworm control and one Cry protein for control of several lepidopteran pests; they also feature herbicide tolerance (to either glyphosate or glufosinate). Slightly more than 50 years has passed since Vernon Stern and his University of California entomology colleagues published (1959) their seminal paper on the integrated control concept, laying the foundation for modern pest management (IPM) programs. To assess the relevance of traditional IPM concepts within a transgenic agroecosystem, commercial maize producers were surveyed at a series of meetings in 2009 and 2010 regarding their perceptions on their use of Bt hybrids and resistance management. Special attention was devoted to two insect pests of corn, the European corn borer and the western corn rootworm. A high percentage of producers who participated in these meetings planted Bt hybrids in 2008 and 2009, 97 and 96.7%, respectively. Refuge compliance in 2008 and 2009, as mandated by the U.S. Environmental Protection Agency (EPA), was 82 and 75.7%, respectively, for those producers surveyed. A large majority of producers (79 and 73.3% in 2009 and 2010, respectively) revealed that they would, or had, used a Bt hybrid for corn rootworm (Diabrotica virgifera virgifera LeConte) or European corn borer (Ostrinia nubilalis Hübner) control even when anticipated densities were low. Currently, the EPA is evaluating the long-term use of seed blends (Bt and non-Bt) as a resistance management strategy. In 2010, a large percentage of producers, 80.4%, indicated they would be willing to use this approach. The current lack of integration of management tactics for insect pests of maize in the U.S. Corn Belt, due primarily to the escalating use of transgenic Bt hybrids, may eventually result in resistance evolution and/or other unforeseen consequences.

12. Domingo J L and Bordonaba JG (2011): A literature review on the safety assessment of genetically modified plants. Environment International 37. 734-42.

In recent years, there has been a notable concern on the safety of genetically modified (GM) foods/plants, an important and complex area of research, which demands rigorous standards. Diverse groups including consumers and environmental Non Governmental Organizations (NGO) have suggested that all GM foods/plants should be subjected to long-term animal feeding studies before approval for human consumption. In 2000 and 2006, we reviewed the information published in international scientific journals, noting that the number of references concerning human and animal toxicological/health risks studies on GM foods/plants was very limited. The main goal of the present review was to assess the current state-of-the-art regarding the potential adverse effects/safety assessment of GM plants for human consumption. The number of citations found in databases (PubMed and Scopus) has dramatically increased since 2006. However, new information on products such as potatoes, cucumber, peas or tomatoes, among others was not available. Corn/maize, rice, and soybeans were included in the present review. An equilibrium in the number research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was currently observed. Nevertheless, it should be noted that most of these studies have been conducted by biotechnology companies responsible of commercializing these GM plants. These findings suggest a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies. All this recent information is herein critically reviewed.
http://www.ncbi.nlm.nih.gov/pubmed/?term=A+literature+review+on+the+safety+assessment+of+genetically+modified+plants.+Environment+International+37.+734-42

13. Diels J, Cunha M, Manaia C, Sabugosa-Madeira B and Silva M (2011): Association of financial or professional conflict of interest to research outcomes on health risks or nutritional assessment studies of genetically modified products. Food Policy 36: 197-203.

Since the first commercial cultivation of genetically modified crops in 1994, the rapidly expanding market of genetically modified seeds has given rise to a multibillion dollar industry. This fast growth, fueled by high expectations towards this new commercial technology and shareholder trust in the involved industry, has provided strong incentives for further research and development of new genetically modified plant varieties. Considering, however, the high financial stakes involved, concerns are raised over the influence that conflicts of interest may place upon articles published in peer-reviewed journals that report on health risks or nutritional value of genetically modified food products. In a study involving 94 articles selected through objective criteria, it was found that the existence of either financial or professional conflict of interest was associated to study outcomes that cast genetically modified products in a favorable light ($p = 0.005$). While financial conflict of interest alone did not correlate with research results ($p = 0.631$), a strong association was found between author affiliation to industry (professional conflict of interest) and study outcome.

14. Seralini G-E, Mesnage R, Clair E, Gress S, de Vendômois JS and Cellier D (2011): Genetically modified crops safety assessments: present limits and possible improvements. Environmental Sciences Europe 23:10

We reviewed 19 studies of mammals fed with commercialized genetically modified soybean and maize which represent, per trait and plant, more than 80% of all environmental genetically modified organisms (GMOs) cultivated on a large scale, after they were modified to tolerate or produce a pesticide. We have also obtained the raw data of 90-day-long rat tests following court actions or official requests. The data obtained include biochemical blood and urine parameters of mammals eating. Several convergent data appear to indicate liver and kidney problems as end points of GMO diet effects in the above-mentioned experiments. This was confirmed by our meta-analysis of all the in vivo studies published, which revealed that the kidneys were particularly affected, concentrating 43.5% of all disrupted parameters in males, whereas the liver was more specifically disrupted in females (30.8% of all disrupted parameters). The 90-day-long tests are insufficient to evaluate chronic toxicity, and the signs highlighted in the kidneys and livers could be the onset of chronic diseases. However, no minimal length for the tests is yet obligatory for any of the GMOs cultivated on a large scale, and this is socially unacceptable in terms of consumer health protection. We are suggesting that the studies should be improved and prolonged, as well as being made compulsory, and that the sexual hormones should be assessed too, and moreover, reproductive and multigenerational studies ought to be conducted too.

15. Hartmut Meyer (2011): Systemic risks of genetically modified crops: the need for new approaches to risk assessment. Environmental Sciences Europe 23:7

Since more than 25 years, public dialogues, expert consultations and scientific publications have concluded that a comprehensive assessment of the implications of genetic engineering in agriculture and food production needs to include health, environmental, social and economical aspects, but only very few legal frameworks allow to assess the two latter aspects. This article aims to explain the divergence between societal debate and biosafety legislation and presents approaches to bring both together. The article reviews the development of biosafety regulations in the USA and the EU, focussing on diverging concepts applied for assessing the risks of genetically modified organisms (GMOs). The dominant environmental risk assessment methodology has been developed to answer basic questions to enable expedient decision making. As a first step, methodologies that take into account complex environmental and landscape aspects should be applied. Expanding the scope of risk assessment, more holistic concepts have been developed, for example the Organisation for Econonomic Co-operation and Development (OECD) concept of systemic risks which includes socio-economic aspects. International bodies as the OECD, the Convention on Biological Diversity (CBD) and the European Union (EU) have developed the Strategic Environmental Assessment (SEA) as an instrument that includes the additional aspects of risk assessment as demanded by many stakeholders. Interestingly, there had been no attempts yet to link the existing frameworks of GMO risk assessment and SEA. It is recommended to adapt current models of SEA to assess the systemic risks of GMOs. It is also suggested to revise the EU GMO legislation to promote the inclusion of SEA elements.

16. Esha Shah, 'Science' in the Risk Politics of Bt brinjal (2011): Economic & Political Weekly, July 30, 2011, Vol xlvi no 31.

Drawing on the literature on controversies, especially on the health risk assessment of genetically modified organisms in Europe, and long-standing debates in science and technology studies, this article argues that science-based risk assessment has inherent limitations, however rigorous, independent, and peer reviewed the work may be. In this context, the debate on Bt brinjal needs to broaden its frame from science-based assessment of consequences to evaluate society-oriented causes and objectives. We need to ask questions such as: What kind of society do we wish to live in? What kind of science and technology do we want? Who sets the agenda for science and technology development and who controls the science and technological decisions?

17. Hilbeck A, Meier M, Römbke J, Jänsch S, Teichmann H, Tappeser B. (2011): Environmental risk assessment of genetically modified plants - concepts and controversies. Environmental Sciences Europe. 2011; 23(13)

Based on the EU ERA framework, we present an improved ERA concept that is system oriented with the GM plant at the centre and integrates a procedure for selection of testing organisms that do occur in the receiving environment. We also propose a hierarchical testing scheme from laboratory studies to field trials and we illustrate the outcomes for three different crop case examples. Our proposed concept can alleviate a number of deficits identified in the current approach to ERA of GM plants. It allows the ERA to be tailored to the GM plant case and the receiving environment.

18. Duan JJ, Lundgren JG, Naranjo S, Marvier M. (2010): Extrapolating non-target risk of Bt crops from laboratory to field. Biol Lett. 6(1): 74-77

The tiered approach to assessing ecological risk of insect-resistant transgenic crops assumes that lower tier laboratory studies, which expose surrogate non-target organisms to high doses of insecticidal proteins, can detect harmful effects that might be manifested in the field. To test this assumption, we performed meta-analyses comparing results for non-target invertebrates exposed to Bacillus thuringiensis (Bt) Cry proteins in laboratory studies with results derived from independent field studies examining effects on the abundance of non-target invertebrates. For Lepidopteran-active Cry proteins, laboratory studies correctly predicted the reduced field abundance of non-target Lepidoptera. However, laboratory studies incorporating tri-trophic interactions of Bt plants, herbivores and parasitoids were better correlated with the decreased field abundance of parasitoids than were direct-exposure assays. For predators, laboratory tri-trophic studies predicted reduced abundances that were not realized in field studies and thus overestimated ecological risk. Exposure to Coleopteran-active Cry proteins did not significantly reduce the laboratory survival or field abundance of any functional group examined. Our findings support the assumption that laboratory studies of transgenic insecticidal crops show effects that are either consistent with, or more conservative than, those found in field studies, with the important caveat that laboratory studies should explore all ecologically relevant routes of exposure.

19. Seetharam S (2010): Should the Bt brinjal controversy concern healthcare professionals and bio-ethicists?. Indian J Med Ethics. Jan-Mar 7 (1)

The Genetic Engineering Approval Committee's approval of Bt brinjal, the first genetically modified crop for human consumption in India, has sparked off protests across the country. This article questions the so-called benefits of GM crops and highlights some major concerns. These include: inadequately addressed health and environmental risks, inadequate safety guidelines, a lack of transparency in sharing test data, the implications to seed sovereignty of farmers and the lack of informed choice for consumers. Some concerns about field testing by Mahyco, the developer of Bt-brinjal, and the process of evaluation by GEAC remain unresolved. With inadequate information about the crop's long-term safety, a precautionary approach is advocated before national policy allows commercial release of the seeds. A fair process is also needed in the public consultations being proposed by the minister of state for environment and forests. In addition to issues of procedural justice, a basic ethical question remains: do humans have a right to dominate the land and make expendable those creatures that they deem "undesirable"?

www.ncbi.nlm.nih.gov/pubmed/20166287

20. Gillam C. (2010): West Virginia investigating Monsanto for consumer fraud. Reuters, June 25.

http://www.reuters.com/article/idUSN2515475920100625explosionthreatens-monsanto-heartlands-genetically-modified-US-crops

21. García L. (2010): Argentina wins Monsanto GM patent dispute in Europe. SciDev.net, July 21 http://www.scidev.net/en/news/argentina-wins-monsanto-gm-patent-dispute-in-europe.html along with

22. GRAIN (2004): Monsanto's royalty grab in Argentina. October.

http://www.grain.org/articles/?id=4 and

23. Nellen-Stucky, R., Meienberg, F. (2006) Harvesting royalties for sowing dissent? Monsanto's campaign against Argentina's patent policy. GRAIN, October.

http://www.grain.org/research/contamination.cfm?id=379

24. Lang, A & Otto, M. (2010): A synthesis of laboratory and field studies on the effects of transgenic Bacillus thuringiensis (Bt) maize on non-target Lepidoptera. Entomologia Experimentalis et Applicata 135 (2): 121134.

One of the major applications of transgenic crops in agriculture are the so-called Bacillus thuringiensis Berliner (Bt) plants, in particular Bt maizes, which produce insecticidal Cry proteins that target specific orders, such as the Lepidoptera or Coleoptera. We reviewed publications that reported on the direct toxic effects of Bt-maize and/or Cry proteins of current Bt-maize events on larvae of non-target butterflies and moths (Lepidoptera). In total, 20 peer-reviewed publications were identified, of which 16 papers contributed laboratory-based data and seven field-based data. An adverse effect on caterpillars was recorded in 52% of all laboratory-based and in 21% of all field-based observations. The variables most often studied and having the highest occurrence of effects were larval survival, body mass, and developmental time. Parameters of the adult stage were under-represented in the studies. Overall, 11 lepidopteran species were tested. The majority of the studies originated from the USA, with the Monarch butterfly being the most studied, whereas other species and other parts of the world were widely neglected. Laboratory experiments were often run under unrealistic conditions from an ecological point of view. Although the papers we reviewed indicated a potential hazard for Lepidoptera that are exposed to and feed on lepidopteran-specific Bt-maize pollen, a general conclusion on the level of risk for butterflies and moths cannot as yet be drawn. A comprehensive risk characterization would require thorough hazard identification, exposure assessment, and impact assessment. However, our review showed that even the basic level of hazard characterization is as yet incomplete. Reasons for this are the still-limited numbers of publications and concurrent lack of knowledge, the restriction of data to only a few species, the over-representation of North American species, and the identified limitations of both laboratory and field experiments. The findings of this review suggest that more realistic, ecologically meaningful, and detailed experiments and analyses are crucial to improve the present assessment of Bt-maize cultivation effects on Lepidoptera.

25. Waltz, E. (2009): Under wraps. Nature Biotechnology 27: 880.

"Are the crop industry's strong-arm tactics and close-fisted attitude to sharing seeds holding back independent research and undermining public acceptance of transgenic crops? Emily Waltz investigates".

http://www.emilywaltz.com/Biotech_crop_research_restrictions_Oct_2009.pdf

26. Waltz, E. (2009): Battlefield. Nature 461: 27-32.

Papers suggesting that biotech crops might harm the environment attract a hail of abuse from other scientists. Emily Waltz asks if the critics fight fair.

http://www.nature.com/news/2009/090902/full/461027a.html

27. Glover, D. (2009): Undying Promise: Agricultural Biotechnology's Pro-poor Narrative, Ten Years on, STEPS Working Paper 15, Brighton: STEPS Centre

Many people and organisations have sought to promote genetically modified (GM, transgenic) crops as a 'pro-poor' technology. However, developing-country farmers' experiences with GM crops have been mixed. Some farmers have certainly benefited, but others have not. Predictably, the performance and impacts of transgenic crops depend critically on a range of technical, socio-economic and institutional factors. By themselves, genetically modified seeds are not enough to guarantee a good harvest or to create a sustainable and productive farm livelihood. In spite of this emerging picture of complex and differentiated impacts, the simplistic narrative of GM crops as a uniformly 'pro-poor' technology has proved to be extraordinarily resilient. Why has it persisted? Part of the reason is that a substantial number of econometric studies have claimed to demonstrate that GM crops are a technological and economic success in the developing world. But methodological and presentational flaws in those studies have created a distorted picture of both the performance and the impacts of GM crops in smallholder farming contexts. This has seriously distorted public debate and impeded the development of sound, evidence-based policy. This paper examines the hidden assumptions that have shaped both the pro-poor claims on behalf of GM crops and the methods that have been used to evaluate them. Those assumptions have involved the radical simplification of the complex agronomic and livelihood contexts into which GM crops have been inserted. They have thus undermined the usefulness and relevance of the information which has been presented to both farmers and policy makers.

http://www.ids.ac.uk/files/dmfile/BtCottonweb.pdf

28. Séralini GE, de Vendomois JS, Cellier D, et al. (2009): How subchronic and chronic health effects can be neglected for GMOs, pesticides or chemicals. Int J Biol Sci. 5(5): 438-443.

Chronic health effects are increasing in the world such as cancers, hormonal, reproductive, nervous, or immune diseases, even in young people. During regulatory toxicological subchronic tests to prevent these on mammalian health, prior commercialization of chemicals, including pesticides and drugs, or GMOs, some statistically significant findings may be revealed. This discussion is about the need to investigate the relevant criteria to consider those as biologically significant. The sex differences and the non linear dose or time related effects should be considered in contrast to the claims of a Monsanto-supported expert panel about a GMO, the MON 863 Bt maize, but also for pesticides or drugs, in particular to reveal hormone-dependent diseases and first signs of toxicities.

29. Johnson, W.G., Owen, M.D.K., Kruger, G.R., Young, B.G., Shaw, D.R., Wilson, R.G., Wilcut, J.W., Jordan, D.L. & Weller, S.C. (2009): US farmer awareness of glyphosate-resistant weeds and resistance management strategies. Weed Technology 23: 308312

A survey of farmers from six U.S. states (Indiana, Illinois, Iowa, Nebraska, Mississippi, and North Carolina) was conducted to assess the farmers' views on glyphosate-resistant (GR) weeds and tactics used to prevent or manage GR weed populations in genetically engineered (GE) GR crops. Only 30% of farmers thought GR weeds were a serious issue. Few farmers thought field tillage and/or using a non-GR crop in rotation with GR crops would be an effective strategy. Most farmers did not recognize the role that the recurrent use of an herbicide plays in evolution of resistance. A substantial number of farmers underestimated the potential for GR weed populations to evolve in an agroecosystem dominated by glyphosate as the weed control tactic. These results indicate there are major challenges that the agriculture and weed science communities must face to implement long-term sustainable GE GR-based cropping systems within the agroecosystem.

30. Magana-Gomez JA and de la Barca AM (2009): Risk assessment of genetically modified crops for nutrition and health. Nutr Rev. 67 (1)

The risk assessment of genetically modified (GM) crops for human nutrition and health has not been systematic. Evaluations for each GM crop or trait have been conducted using different feeding periods, animal models, and parameters. The most common result is that GM and conventional sources induce similar nutritional performance and growth in animals. However, adverse microscopic and molecular effects of some GM foods in different organs or tissues have been reported. Diversity among the methods and results of the risk assessments reflects the complexity of the subject. While there are currently no standardized methods to evaluate the safety of GM foods, attempts towards harmonization are on the way. More scientific effort is necessary in order to build confidence in the evaluation and acceptance of GM foods.

31. Do seed companies control GM crop research? Editorial, Scientific American, August (2009)

Research on genetically modified seeds is still published, of course. But only studies that the seed companies have approved ever see the light of a peer-reviewed journal. In a number of cases, experiments that had the implicit go-ahead from the seed company were later blocked from publication because the results were not flattering. "It is important to understand that it is not always simply a matter of blanket denial of all research requests, which is bad enough," wrote Elson J. Shields, an entomologist at Cornell University, in a letter to an official at the Environmental Protection Agency (the body tasked with regulating the environmental consequences of genetically modified crops), "but selective denials and permissions based on industry perceptions of how 'friendly' or 'hostile' a particular scientist may be toward [seed-enhancement] technology."

Although we appreciate the need to protect the intellectual property rights that have spurred the investments into research and development that have led to agritech's successes, we also believe food safety and environmental protection depend on making plant products available to regular scientific scrutiny. Agricultural technology companies should therefore immediately remove the restriction on research from their end-user agreements. Going forward, the EPA should also require, as a condition of approving the sale of new seeds, that independent researchers have unfettered access to all products currently on the market. The agricultural revolution is too important to keep locked behind closed doors.

http://www.scientificamerican.com/article.cfm?id=do-seed-companiescontrol-gm-crop-research

32. Deguine J-P, Ferron P and Russell D. (2008): Sustainable pest management for cotton production. A review. Agron. Sustain. Dev. 28 (1): 113137

Cotton cultivation, often highlighted for its excessive consumption of plant protection products, is taken as a model to illustrate the development of the ideas and practices of crop protection over the last 50 years. Cotton is grown in 69 countries on 30-35 million hectares and the production exceeded 20 million tones of lint in recent years. Despite the continual improvement in the performance of chemical control strategies, harvest losses remain very high, of about 30%. The largest consumer of pesticides in the world, the cotton production system has the advantage of having been an experimental model for many crop protection programmes under various agronomic conditions and in the presence of diverse pest complexes. Without attempting an exhaustive bibliography, this review explores how and why the ideas underlying crop protection have significantly evolved since the advent of synthetic pesticides. After a spectacular demonstration of yield growth through the application of chemical control, cotton production was rapidly confronted by the secondary effects of this control. These included the appearance of evolved insecticide resistance and the appearance of new damage caused by pests considered up to then as of only secondary importance. In extreme cases, the economic viability of the production systems themselves have been compromised following increases in the application rate and frequency of insecticidal treatments. In general, harvest losses have remained high despite the constantly improving technical performance of pest control chemicals. Two models of the future of crop protection can be drawn: total pest management which involves the eradication of pests, and integrated pest management (IPM), which aims at the management of pest populations below economic thresholds by a mixture of chemical control and a suite of alternative control measures. The first method, total pest management is limited in agricultural systems to particular cases in which the pest in question has no significant alternate hosts in the vicinity of the crop system. On the other hand, the application of IPM is constrained both by

the difficulties in exploiting the concept of an `intervention threshold' and by the limitations of many of the specific non-chemical techniques proposed, but does have the advantage of taking into consideration the full pest complex in a cropping system. In practice, it has been a calendar schedule, largely of insecticidal treatments, established on the basis of earlier local observations which has been most widely adopted by growers. This strategy has produced significant improvements in production in the cotton producing countries of francophone Africa and elsewhere. This has led to area-wide integrated pest management which takes into account the potential for natural factors to regulate populations in a specific region. In cotton production, biological control by introduction and acclimation of beneficial arthropods has not been notably successful because of the difficulty of developing a suite of beneficial organisms capable of responding effectively to the diversity of pests in the system, the annual nature of the crop, and the disrupting effects of chemical control measures directed against the remaining pests. Only inundative biological control has had significant success and then in particular cases where the pressure of chemical insecticides has been reduced. More benefit is to be obtained from the active conservation of the indigenous fauna of beneficial organisms. In spite of an increased general environmental awareness, in practice it has been the growth of evolved resistance to pesticides which has had the dominant role in constraining the growers to a more rational use of control strategies. These can be illustrated by the development of window strategies for control measures across the growing season, initially in Australia. The reduction in chemical control treatments made possible by the efficacy of genetically modified cotton has shown the positive role that indigenous natural enemies can play. At the same time, however, there has been a growth in the importance of pest species which are unaffected by Bt toxins. For example, the sucking pests are progressively coming to displace the vegetative and fruit feeding caterpillars as key pests of Bt cotton. Taking into account the spatio-temporal dimension of natural population regulatory factors has led to changes in agricultural practices and production systems. In cotton, for example, production systems maintaining permanent ground cover, are

having increasing success. Intercropping and trap cropping have been favourable to the maintenance of beneficial arthropod complexes and unfavourable to the growth of pest populations. This new design context for crop protection in general and for cotton in particular, in applying the principles of agroecology, moves towards the concept of a truly sustainable agriculture. This implies a change of strategy towards a total systems approach to sustainable pest management, characterised by a movement from a paradigm of pest control field-by-field, through farm-by-farm and agroecosystem-by-agroecosystem, to a landscape by landscape approach.

33. Kroghsbo S, Madsen C, Poulsen M, et al. (2008): Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. Toxicology. 245(1-2): 24-34.

As part of the SAFOTEST project the immunmodulating effect of Cry1Ab protein from Bacillus thuringiensis (Bt) and PHA-E lectin from kidney bean (Phaseolus vulgaris erythroagglutinin) was examined in 28- and 90-day feeding studies in Wistar rats. PHA-E lectin was chosen as positive control. Rats were fed control rice, transgenic rice expressing Cry1Ab protein or PHA-E lectin, or transgenic rice spiked with the purified recombinant protein. Total immunoglobulin levels, mitogen-induced cell proliferation, T-dependent antibody response to sheep red blood cells and the antigen-specific antibody response in serum were examined at the end of the studies. A dose-dependent increase in mesenteric lymph node weight and total immunoglobulin A was seen when feeding PHA-E transgenic rice alone or spiked with 0.1% purified PHA-E lectin for 90 days indicating a local effect of PHA-E in the intestine. No adverse effects of Cry1Ab protein were found. An anti-PHA-E and anti-Cry1Ab antibody response was induced both after inhalation (control groups) and after inhalation/ingestion (groups fed recombinant protein alone or together with transgenic rice). In conclusion, only PHA-E lectin was found to have an immunomodulating effect when feeding rats for 90 days with approximately 70mg PHA-E/kg bodyweight per day. As both PHA-E lectin and Cry1Ab protein were capable of inducing an antigen-specific antibody response it is important to make careful considerations when designing future animal studies to avoid intake of proteins from the other groups by inhalation as well as to examine the sensitization and elicitation potential of 'foreign' proteins before introduction to the world market.

34. Binimelis R (2008): Coexistence of plants and coexistence of farmers: is an individual choice possible? Journal of Agricultural and Environmental Ethics. DOI10.1007/s10806-008-9099-4

The introduction of genetically modified organisms (GMOs) in Europe has been characterized by controversy. In 2002, the European Union introduced the concept of "coexistence" as a compromise solution that, through the establishment of science-based technical measures, should allow the market to operate freely while reducing policy conflicts on GMOs. However, the concept remains highly contested and the technical measures difficult to apply. This paper presents qualitative research on the conceptualization and implementation of the coexistence framework in two regions of Spain (Catalonia and Aragon), where 42% and 55% of maize was GM in 2006, respectively. In this context, the concept of coexistence and its proposed implementation both fail to resolve previous conflicts and actually work to generate new ones through the individualization of choice and impacts. Considerations of the social conditions in which the technology and the management measures are implemented were not taken into account. This resulted in the promotion of biotechnological agriculture over other alternatives.

http://rd.springer.com/article/10.1007/s10806-008-9099-4

35. Beintema, N. et al. (2008): International Assessment of Agricultural Knowledge, Science and Technology for Development: Global Summary for Decision Makers (IAASTD).

http://www.agassessment.org/index.cfm?Page=IAASTD%20Reports&ItemID=2713

36. Martin Paul Krayer von Krauss, Mathias Kaiser, Vibeke Almaas, Jeroen van der Sluijs, Penny Kloprogge (2008): Diagnosing and prioritising uncertainties according to their relevance for policy: The case of transgene silencing. Science of the Total Environment. 390 (1): 23-34.

Uncertainty often becomes problematic when science is used to support decision making in the policy process. Scientists can contribute to a more constructive approach to uncertainty by making their uncertainties transparent. In this article, an approach to systematic uncertainty diagnosis is demonstrated on the case study of transgene silencing and GMO risk assessment. Detailed interviews were conducted with five experts on transgene silencing to obtain quantitative and qualitative information on their perceptions of the uncertainty characterising our knowledge of the phenomena. The results indicate that there are competing interpretations of the causeeffect relationships leading to gene silencing (model structure uncertainty). In particular, the roles of post-transcriptional gene silencing, position effects, DNADNA interactions, direct-repeat DNA structures, recognition factors and dsRNA and aberrant zRNA are debated. The study highlights several sources of uncertainty beyond the statistical uncertainty commonly reported in risk assessment. The results also reveal a discrepancy between the way in which uncertainties would be prioritized on the basis of the uncertainty analysis conducted, and the way in which they would be prioritized on the basis of expert willingness to pay to eliminate uncertainty. The results also reveal a diversity of expert opinions on the uncertainty characterizing transgene silencing. Because the methodology used to diagnose uncertainties was successful in revealing a broad spectrum of uncertainties as well as a diversity of expert opinion, it is concluded that the methodology used could contribute to increasing transparency and fostering a critical discussion on uncertainty in the decision making process.

http://www.sciencedirect.com/science/article/pii/S0160791X13000080

37. Stone GD (2007): Agricultural deskilling and the spread of genetically modified cotton in Warangal. Current Anthropology. Vol. 48 (1): 67-103.

Warangal District, Andhra Pradesh, India, is a key cotton-growing area in one of the most closely watched arenas of the global struggle over genetically modified crops. In 2005 farmers adopted India's first genetically modified crop, Bt cotton, in numbers that resemble a fad. Various parties, including the biotechnology firm behind the new technology, interpret the spread as the result of farmer experimentation and management skill, alluding to orthodox innovation-diffusion theory. However, a multiyear ethnography of Warangal cotton farmers shows a striking pattern of localized, ephemeral cotton seed fads preceding the spread of the genetically modified seeds. The Bt cotton fad is symptomatic of systematic disruption of the process of experimentation and development of management skill. In fact, Warangal cotton farming offers a case study in agricultural deskilling, a process that differs in fundamental ways from the better-known process of industrial deskilling. In terms of cultural evolutionary theory, deskilling severs a vital link between environmental and social learning, leaving social learning to propagate practices with little or no environmental basis. However, crop genetic modification is not inherently deskilling and, ironically, has played a role in reinvolving farmers in Gujarat in the process of breeding.

38. Poulsen M, Kroghsbo S, Schroder M, et al. (2006): A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin Galanthus nivalis (GNA). Food Chem Toxicol.

Genetically modified plants expressing insecticidal traits offer a new strategy for crop protection, but at the same time present a challenge in terms of food safety assessment. The present 90-day feeding study was designed to assess the safety of a rice variety expressing the snowdrop Galanthus nivalis lectin (GNA lectin), and forms part of a EU-funded project where the objective has been to develop and validate sensitive and specific methods to assess the safety of genetically modified foods. Male and female Wistar rats were given a purified diet containing either 60% genetically modified or parental rice for 90 days. This corresponds to a mean daily GNA lectin intake of approximately 58 and 67mg/kg body weight for males and females, respectively. Prior to the animal study comprehensive analytical characterization of both rice materials was performed. The chemical analyses showed a number of statistically significant differences, with the majority being within the ranges reported in the literature. In the animal study a range of clinical, biological, immunological, microbiological and pathological parameters were examined. A number of significant differences were seen between groups fed the two diets, but none of them were considered to be adverse. In conclusion, the design of the present animal study did not enable us to conclude on the safety of the GM food. Additional group(s) where the expressed gene products have been spiked to the diet should be included in order to be able to distinguish whether the observed effects were due to the GNA lectin per se or to secondary changes in the GM rice.

39. Andow, D.A. & Zwahlen, C. (2006): Assessing environmental risks of transgenic plants. Ecology Letters 9: 196-214.

By the end of the 1980s, a broad consensus had developed that there were potential environmental risks of transgenic plants requiring assessment and that this assessment must be done on a case-by-case basis, taking into account the transgene, recipient organism, intended environment of release, and the frequency and scale of the intended introduction. Since 1990, there have been gradual but substantial changes in the environmental risk assessment process. In this review, we focus on changes in the assessment of risks associated with non-target species and biodiversity, gene flow, and the evolution of resistance. Non-target risk assessment now focuses on risks of transgenic plants to the intended local environment of release. Measurements of gene flow indicate that it occurs at higher rates than believed in the early 1990s, mathematical theory is beginning to clarify expectations of risks associated with gene flow, and management methods are being developed to reduce gene flow and possibly mitigate its effects. Insect pest resistance risks are now managed using a high-dose/refuge or a refuge-only strategy, and the present research focuses on monitoring for resistance and encouraging compliance to requirements. We synthesize previous models for tiering risk assessment and propose a general model for tiering. Future transgenic crops are likely to pose greater challenges for risk assessment, and meeting these challenges will be crucial in developing a scientifically coherent risk assessment framework. Scientific understanding of the factors affecting environmental risk is still nascent, and environmental scientists need to help improve environmental risk assessment.

http://onlinelibrary.wiley.com/doi/10.1111/j.1461-0248.2005.00846.x/Abstract

40. Pengue, W. (2005): Transgenic crops in Argentina: the ecological and social debt. Bulletin of Science, Technology and Society 25: 314-322.

There is no doubt that soybean is the most important crop for Argentina, with a planted surface that rose 11,000,000 hectares and a production of around 35,000,000 metric tons. During the 1990s, there was a significant agriculture transformation in the country, motorize by the adoption of transgenic crops (soybean, maize, and cotton) under the no-tillage system. The expansion of this model has been spread not only in the Pampas but also in very rich areas with high biodiversity, opening a new agricultural border to important eco-regions like the Yungas, Great Chaco, and the Mesopotamian Forest. Transgenic cropping is a powerful technology. This produced relevant transformations over the environment and society where it is allowed. Migration, concentration of agribusiness, and loss of food sovereignty are some of the social results. Landscape transformation in the rural sector is evident, and the appearance of tolerance weeds to glyphosate is a reality. Nutrient depletion, soilstructure degradation, potential desertification, and loss of species are other consequences on the environmental level.

www.bch.cbd.int/database/attachedfile.aspx?id=1538

41. Duke, S.O. (2005): Taking stock of herbicide-resistant crops ten years after introduction. Pest Management Science 61: 211218

Since transgenic, bromoxynil-resistant cotton and glufosinate-resistant canola were introduced in 1995, planting of transgenic herbicide-resistant crops has grown substantially, revolutionizing weed management where they have been available. Before 1995, several commercial herbicide-resistant crops were produced by biotechnology through selection for resistance in tissue culture. However, non-transgenic herbicide-resistant crops have had less commercial impact. Since the introduction of glyphosate-resistant soybean in 1996, and the subsequent introduction of other glyphosate-resistant crops, where available, they have taken a commanding share of the herbicide-resistant crop market, especially in soybean, cotton and canola. The high level of adoption of glyphosate-resistant crops by North American farmers has helped to significantly reduce the value of the remaining herbicide market. This has resulted in reduced investment in herbicide discovery, which may be problematic for addressing future weed-management problems. Introduction of herbicide-resistant crops that can be used with selective herbicides has apparently been hindered by the great success of glyphosate-resistant crops. Evolution of glyphosate-resistant weeds and movement of naturally resistant weed species into glyphosate-resistant crop fields will require increases in the use of other herbicides, but the speed with which these processes compromise the use of glyphosate alone is uncertain. The future of herbicide-resistant crops will be influenced by many factors, including alternative technologies, public opinion and weed resistance. Considering the relatively few recent approvals for field testing new herbicide-resistant crops and recent decisions not to grow glyphosate-resistant sugarbeet and wheat, the introduction and adoption of herbicide-resistant crops during the next 10 years is not likely to be as dramatic as in the past 10 years.

42. Andow, D.A. and A. Hilbeck. (2004): Science-based risk assessment for non-target effects of transgenic crops. Bioscience 54 (7): 637-649

Nontarget risk assessment for transgenic crops should be case specific, depending on the plant, the transgene, and the intended release environment. We propose an ecological risk-assessment model that preserves the strengths and avoids the deficiencies of two other commonly used models, the ecotoxicology and nonindigenous-species models. In this model, locally occurring nontarget species are classified into groups according to their ecological function. Within each group, ecological criteria are used to select the species that are most likely to be affected by the transgenic crop. Initial experimental assessments are conducted in the laboratory and consist of two kinds of test: toxicity tests using purified transgene product, and whole-plant tests using intact transgenic plants. For nontarget natural enemy species, it will also be important to evaluate both direct bitrophic impacts and indirect tritrophic impacts.

43. Freese W and Schubert D (2004): Safety testing and regulation of Genetically Engineered foods. Biotechnology and Genetic Engineering Reviews. Vol 21. 299-324

We have described the US regulatory system for GE foods, and with specific examples pointed out serious deficiencies in both regulatory oversight and corporate testing procedures. It is clear that the US regulatory process must be made mandatory, as well as more stringent and transparent. Any legal obstacles standing in the way of a thorough, mandatory, premarket review process must be overcome, with new statutes specifically designed for genetically engineered foods. Truly sound science must prevail in the debate over genetically engineered foods to ensure the safety of both consumers and the environment. The outline for an initial screening regimen proposed here offers an additional step toward this end.

http://www.saveourseeds.org/downloads/schubert_safety_reg_us_11_2004.pdf

44. Marvier M. (2002): Improving risk assessment for non-target safety of transgenic crops. Ecological Applications 12: 1119-1124

In many countries, government regulations require environmental risk assessment prior to commercial sale and widespread planting of transgenic crops. Here I evaluate the design and statistical rigor of experiments used by industry to assess the safety of transgenic plants for nontarget organisms, as required under U.S. regulations. This review reveals that a few simple improvements in experimental design could greatly increase the rigor and information content of studies required under current regulations. For example, although most experiments were conducted for 14 wk, some of the tested species can live a year or more and could experience much longer periods of exposure. Moreover, the number of replicates used in these studies was generally quite small (usually 26 replicates per treatment), resulting in experiments that had little chance of detecting real effects. Clearly, sample sizes should be bolstered, and nonsignificant results should be accompanied by an analysis of statistical power. In addition, information readily available over the Internet is insufficient for a quantitative assessment of a transgenic crop's safety. Improved access to information regarding the details of risk assessment studies could greatly increase the public's ability to evaluate industry's claims of safety.

http://www.sacbee.com/static/live/news/projects/biotech/imgs/grfx_c2_5.pdf

45. Penninks AH, Knippels LM. (2001): Determination of protein allergenicity: studies in rats. Toxicol Lett. 120(1-3): 171-180

For the safety evaluation of genetically engineered crops the potential allergenicity of the newly introduced protein(s) has become an important issue. There is, however, no universal and reliable test system for the evaluation of the allergenic potency of food products. The best known allergy assessment proposal is the careful stepwise process using the IFBC/ILSI decision tree. Unfortunately, the described tests are not always conclusive, especially if the gene source coding for the protein has no history of dietary use and/or an unknown history in terms of allergenicity. The further testing warranted should in particular be focused on the prediction of the sensitizing potential of the novel protein, for which animal models are considered to be needed. In this paper the results are summarized of a promising food allergy model developed in Brown Norway (BN) rats. The results demonstrate that BN rats can be sensitized orally to the various allergenic food proteins tested, resulting in significant antigen-specific IgE responses, without the use of adjuvants. Upon oral challenge of previously sensitized animals, local and systemic immune-mediated effects, such as increased gastrointestinal permeability and decreased breathing frequency and blood pressure, could also be observed.

SOME UNPUBLISHED BUT IMPORTANT PAPERS

1. Seralini, G-E (2009): Effects on health and environment of transgenic (or GM) Bt brinjal:

www.criigen.org/SiteFr//images/stories/Dossiers/Divers/btbrinjalges_%25200109.pdf

The dossiers submitted by Mahyco in support of their application for commercialisation of genetically modified (GM) Bt brinjal raise serious concerns. Most of these are not signed by researchers that have performed the tests on pages where they should be (signature frames empty), and could be considered as non valid. Bt brinjal has been modified to produce an unknown chimeric insecticide toxin containing Cry1Ab and Cry1Ac modified sequences. In the toxicity tests on target and non-target insects, this chimeric toxin has not been used but instead, an improper Cry1Ac toxin was used because this control was easier. This could also make these tests not valid. Moreover, Bt brinjal produces into the vegetable cells a protein inducing resistance towards at least kanamycin, a well known antibiotic. This is typical of the first generation of GMOs which have been made without consideration of the problem. Antibiotic resistance is recognized to be a major health problem because of the growing development in the environment and bodies of antibiotic resistance genes. It is very inappropriate to consider commercialising a food containing an antibiotic resistance gene since several modern biotechnology companies have already developed transgenic plants without this kind of marker genes. It is possible that Mahyco has bought an old unused GMO technology to Monsanto Company. Bt brinjal has not been properly tested at a safety or an environmental point of view. However in feeding trials, numerous significant differences were noted compared to the best corresponding non-Bt controls: Bt brinjal appears to contain 15% less kcal/100 g, have a different alkaloïd content, and 16-17 mg/kg Bt insecticide toxin poorly characterized for side effects, and produced by the plant genetically modified for this. Parameters affected in animals fed with this GMO are in blood cells or chemistry, but in different manners according to the period of measurement during the study or the sex: in goats

prothrombin time is modified, and biochemical parameters such as total bilirubin and alkaline phosphatase are also changed, as well as feed consumption and wei ht gain. For rabbits less consumption was noted and also prothrombin time modification, higher bilirubin in some instances, albumin, lactose dehydrogenase and the hepatic markers alanine and aspartate aminotransferases. Sodium levels were also modified, as well as glucose, platelet count, mean corpuscular haemoglobin concentration and haematocrit value. In cows milk production and composition were 10- 14% changed. There was more milk and more roughage dry matter intake like if the animals were treated by a hormone. Rats GM-fed had diarrhoea, higher water consumption, liver weight decrease as well as relative liver to body weight ratio decrease. Feed intake was modified in broiler chickens as well as glucose in some instances. Average feed conversion and efficiency ratios are changed in GM-fed fishes. All that makes a very coherent picture of Bt brinjal that is potentially unsafe for human consumption. It will be also potentially unsafe to eat animals with these problems, having eaten GMOs. These differences are most often ot reported in the summaries of the different experiments but are in the raw data. These differences were, when discussed, disregarded, often on the grounds that they were within the range of a wide "reference" group (really larger than the real closest control group). This reference group represents a wide range of brinjal types and is not a strict comparison. Other reasons for disregarding the differences were that they did not show linear dose response or time response, or that they were only present in either males or females, but not both. Such declarations that the differences seen are not of biological relevance are not substantiated by the data presented from the feeding trials. Clear significant differences were seen that raise food safety concerns and warrant further investigation. The GM Bt brinjal cannot be considered as safe as its non GM counterpart. Indeed, it should be considered as unsuitable for human and animal consumption. In addition, the longest toxicity tests which are for only 90 days do not assess long-term effects like the development of tumours or cancers. It is almost impossible through measurements of toxicity to a few species of non-target organisms to get

a sufficient view of possible harm to complicated ecosystems, which, moreover vary substantially from place to place in India. The experiments on the potential toxicity of GM Bt brinjal to non target organisms (such as butterflies and moths), to beneficial insects and to long-term soil health are woefully inadequate and give no assurances for the environmental safety of growing GM Bt brinjal. Indeed, in many cases the experiments were considered irrelevant (e.g. do not take indirect effects, such as effects up the food chain into account). The gene flow studies assess but not extensively and not in an adequate manner the possibility of GM contaminations, in particular to neighbouring brinjal crops. This neglects other routes of contamination (e.g. by mixing seeds). Based on these tests, Bt brinjal cannot be considered as safe. It is known anyway that natural Bt toxins have never been authorized as such for mammalian consumption. Artificial ones should not be either, before a more serious assessment. Significant effects in comparison to controls are also noticed with other GMOs tolerant to Roundup, and in total with at least four GMOs for which these kinds of tests have been done. These resemble classical side effects of pesticides in toxicology; and these have also been observed for MON810 maize producing a related insecticide which is present in part in the Bt brinjal, Cry1Ab. Brinjal is known to have existed in India for 4000 years. Given that India is also a functional Centre of Origin of brinjal, any release of Bt Brinjal into the environment, poses a significant risk of contamination to sexually compatible wild species and consequent harm to the environment in addition to the contamination of Non-GM varieties. The commercialisation of Bt Brinjal will exacerbate that risk. The release of Bt brinjal for these reasons as well would be a problem. The agreement for Bt brinjal release into the environment, for food, feed or cultures, may present a serious risk for human and animal health and the release should be forbidden

2. Gallagher L (2010): Bt brinjal Event EE1 The scope and adequacy of the GEAC toxicological risk assessment.

www.testbiotech.de/sites/default/files/Report%20Gallagher_2011.pdf

This evaluation of Bt brinjal studies is based on requirements for a rigorous evaluation of food safety for the people of India and their health. Departures from Indian and international published standards for the 14-day and 90-day studies are a cause for concern1. The current food safety studies for Bt brinjal were not conducted in accordance with published standards, did not accurately summarize results, and ignored toxic endpoints for rats fed Bt brinjal: in particular, rats fed Bt brinjal for 78 out of 90 days (only one dose level) experienced:

- ☐ organ and system damage: ovaries at half their normal weight, enlarged spleens with white blood cell counts at 35 to 40 percent higher than normal with elevated eosinophils, indicating immune function changes.
- ☐ toxic effects to the liver as demonstrated by elevated bilirubin and elevated plasma acetylcholinesterase.

Major health problems among test animals were ignored in these reports. The single test dose used was lower than recommended by the Indian protocols. Release of Bt brinjal for human consumption cannot be recommended given the current evidence of toxicity to rats in just 90 days and the studies' serious departures from normal scientific standards.

Unanswered concerns regarding the safety assessment of Bt Brinjal

Neurological function, behavioral effects, reproductive performance and biological resilience of test animals were not evaluated in these studies. Further research based on properly conducted and supervised studies is needed to resolve indications that Bt brinjal may have adverse effects on these clinical endpoints.Dietary equivalence of dried brinjal, dried Bt brinjal and control diets was not addressed. Concentrationsof the new insecticide protein Cry1A(c) were not measured in dried brinjal powder. It is important to know how much of this new protein was actually in the

dried samples fed to the rats, especially since there is data to suggest that Cry1A(c) is at least partially destroyed in laboratory heating conditions. That omission makes it impossible to compare the test diet with insecticide concentrations expected in cooked human food.

3. Andow, D (2010): Bt Brinjal the scope and adequacy of the GEAC environmental risk assessment.

www.indiaenvironmentportal.org.in/files/Bt_Brinjal.pdf

This report evaluates the scope and adequacy of the environmental risk assessment (ERA) for hybrid E-1 Bt brinjal requested by the Genetic Engineering Approval Committee (GEAC) in response to the Maharashtra Hybrid Seeds Company Ltd., Mumbai (Mahyco) application for permission to commercialise hybrid EE-1 Bt brinjal. The assessment is reported in the Report of the Expert Committee (EC-II) on Bt Brinjal Event EE-1. Event EE-1 expresses a genetically engineered crystalline (Cry) protein toxin from the soil bacterium Bacillus thuringiensis Berliner (Bt). The Cry toxin is similar but not identical to Cry1Ac. It is a chimeric protein of Cry1Ac and Cry1Ab, and designated Ccry1A in this report. EE-1 was inserted into improved brinjal hybrids, which are modern cultivars of Solanum melongena L. [Solanaceae: Leptostemonum]. It was developed to control the brinjal fruit and stem borer, Leucinodes orbonalis Guenee [Lepidoptera: Crambidae], which is abbreviated BFSB in the rest of this report. This report does not address the potential risks of hybrid Bt brinjal as human food or animal feed, and does not fully analyse the need for, requirements of, and methodologies for post-commercialisation monitoring of hybrid Bt brinjal. The report is based on an analysis of EC-II, the eight volumes of supporting information to the EC-II, which are referred to as the Dossier in the rest of this report, Supplemental Materials submitted to the GEAC by Mahyco, which comprise 13 technical reports, and publicly available scientific literature on Bt brinjal, BFSB and brinjal.

4. Ramdas, S (2010): Science adulterated. Combat Law. 25 March 2010.

http://www.combatlaw.org/?p=258

There is clearly a total failure and inability of our existing public research institutions and national regulatory bodies (GEAC), to investigate/ test/ rigorously examine, prove or disprove these field observations, preferring to dismiss the reports as "unsubstantiated", "exaggerated, and unscientific", refusing to conduct a single field-based study and instead placing the onus of "proof" on shepherds, farmers and civil society groups who have reported the problem.

The argument that the latest guidelines do not require the suggested new risk assessment tests and hence have been dispensed with negate and ignore the field realities where "non-target organisms" have been affected by the Bt toxin. On the contrary, these unique field experiences and observations, urgently invite new and additional specific regulatory and risk assessment protocols.

Public research institutions are losing their legitimacy as independent institutions working in the interest of the citizens. It is our appraisal that scientists are occupied in lab-based science sponsored by corporations, rather than conducting citizens-based research and apply their science to address and investigate problems that are experienced by farmers in distress.

The inconclusive nature of Bt toxin in cotton and its impact on animals continue to haunt. There is clearly a stress factor that is eliciting a morbid possible allergenic response in the cattle. Is it Bt toxin? Is it some unknown/new toxin? Is it a new allergenic protein? Is it macro/micro mineral imbalances in the Bt cotton plant, (eg excess or deficiency of nitrate, nitrite, selenium etc) as a result of the Bt protein, which elicits a response from the animal? These are questions that the shepherds, farmers and "independent scientists" continue to ask and demand answers. As a first step there is immense need for a comprehensive review of the Bt cotton experience in India, particularly with respect to

health and other bio-safety issues. Tomorrow a bill like the BRAB would definitely qualify that we uniformly get punished, imprisoned, and jailed

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